Polyhydroxyalkanoate production using enriched biomass and acidogenic fermentation products of dairy wastewater and organic food waste

Selin Karaca[a], Nevin Yagci[a,*], Clifford W. Randall[b]

[a]Faculty of Civil Engineering, Environmental Engineering Department, Istanbul Technical University, TR-34469 Maslak, Istanbul, Turkey, Tel. +90 212 285 73 02; Fax: +90 212 285 65 45; emails: yagcin@itu.edu.tr (N. Yagci), slnkaraca@gmail.com (S. Karaca)
[b]Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, Virginia 24061, USA, email: cliff@vt.edu

ABSTRACT

In this study, the optimization of polyhydroxyalkanoates (PHA) production using acidified dairy wastewater (DW) and organic food waste (OFW) as a substrate source was investigated. The study included (a) acclimation of activated sludge in two sequencing batch reactors operated under different operational conditions (feast-famine and anaerobic-aerobic-anoxic) and fed with domestic wastewater only and with volatile fatty acid (VFA) supplement, (b) acidification experiments designed for the initial substrate to inoculum (S/I) ratios of 1, 2.5, 5 and 10 g tCOD/g VSS (tCOD – total chemical oxygen demand and VSS – volatile suspended solids) using DW and OFW, (c) batch experiments for PHA production using each acidified substrate source. The maximum acidification yields were obtained at the S/I ratio of 10 as 51.7% and 46.6% for DW and OFW at 4th d. PHA contents of up to 67.3% and 4.5% of dry cell weight were obtained using VFA obtained from acidification of DW and OFW, respectively. Accordingly, the results showed that acidification of 1000 m³ of DW and one tone of the wet weight of OFW could produce 173 and 20.3 kg of polymer, respectively. This could be attributed to a significant profit when a large amount of DW and OFW is considered.

Keywords: Biomass acclimation; Dairy wastewater; Organic food waste; Acidification; PHA accumulation

1. Introduction

Plastics are often used in the production of short-term or single-use products, although it takes many years to break down in nature after use. For example, in plastic bags with an average use of 12 min, the complete breakdown in nature is about 500 y. Many toxic chemicals are released into the atmosphere [1] when plastics are burned. Besides, plastics occupy large areas in landfills. Although plastics recycling is a principal solution, both the recycling rates and the low quality of recycled plastic are the most critical obstacles to this solution. The uncertainties regarding the sustainability of petroleum-derived plastics, the increases in oil prices, and the environmental concerns led to the emphasis on plastics derived from petroleum derivatives and the shift towards renewable polymer raw material production in recent years.

Bioplastics have been used for nearly 100 y. In recent years, they are used to produce many consumer products such as plastic containers, grocery bags, and food packaging [2,3]. The essential advantages of bioplastics are the lower energy footprint and less pollution on the ecosystem. There is no literature from researchers on potential hazardous additives or compounds in or released from biodegradable plastics during production, usage, and post-usage was found, which enlarged their popularity.

The term bioplastics refer to biodegradable materials derived from renewable sources. Biodegradability and

---

* Corresponding author.

compostability are related to the chemical structure of the polymer, regardless of the raw material. Nowadays, among the bio-based (derived from biomass) plastics, commercially prominent are polylactic acid, polyhydroxyalkanoates (PHA), starch plastics, cellulose esters, and protein-based plastics [4].

PHAs are bio-polyesters, stored within cells as energy storage materials by various microorganisms [5]. The microbial PHA family of polyesters is biodegradable and biocompatible thermoplastics. PHAs have similar properties to synthetic plastics as intracellular storage substances synthesized by various bacteria. Poly-3-hydroxybutyrate (PHB) and poly-3-hydroxyvalerate (PHV) are the most common PHA copolymers used in the production of bioplastics [6]. General characteristics of PHAs are water-insoluble and resistant to hydrolytic degradation, resistant to ultraviolet rays, weak resistance to acids and bases, biocompatibility due to lack of toxic effects suitable for medical applications [7]. Renewable biomass from industry and agriculture or household waste materials can be considered a relatively cheap and non-petroleum-based carbon source for PHA production [8].

A wide variety of microorganisms in activated sludge synthesize PHA and store them as carbon and energy sources [9]. In 1974, Wallen and Rohwedder first conducted a study to develop new technologies to produce biodegradable plastics from activated sludge and found that the PHA content of the activated sludge is 1.3% of the dry weight [10,11]. PHA accumulation by microorganisms can be achieved by making some modifications in conventional biological activated sludge systems. Individual municipal wastewater treatment plants may become suppliers of renewable raw materials of higher value than that of biogas and/or energy and heat [12].

The most common strategies used for the selection of PHA accumulating microorganisms are anaerobic-aerobic (oxygen restricted), microaerophilic-aerobic (oxygen restricted), aerobic dynamic feeding (feast-famine), and nutrient (N, P) restricted [13]. With these strategies, the cost of PHA production, the amount of excess sludge generated in wastewater treatment plants, and consequently, sludge treatment requirements can be considerably reduced [14,15].

PHAs are most typically synthesized in mixed microbial cultures from volatile fatty acids (VFAs) [16–19]. Therefore, the presence of VFAs in wastewater is of great importance in PHA production. Various industrial wastewaters and even organic solid wastes are potential substrates for the low-cost production of PHA due to the high concentrations of VFA produced during fermentation [17,18,20–23]. The significance of pH, the residence time, organic loading rate, and temperature on the acidification process was emphasized for the optimization of the production rate and composition of VFA [24–30].

The dairy industry is generally the largest source of food processing wastewaters. It produces a wide range of pasteurized milk, milk powder, cheese, butter, ice cream, and yogurt. Therefore, the wastewater composition varies considerably depending on the product and the recovery of by-products with economic value. Dairy industry wastewaters are generally rich in organic matter content. Chemical oxygen demand (COD) concentrations in the dairy wastewaters vary in a wide range, such as 2,000 and 68,000 mg/L [31]. In another study, COD concentrations are reported to change in the range of 400–102,100 mg/L [32].

Disposal of food wastes (organic solid wastes), which constitute a significant proportion of domestic solid wastes (50%–60%), has become a significant worldwide problem by causing leachate formation, gas production, groundwater contamination, etc. They are characterized by high moisture content (74%–90%) and biodegradability [33–36]. Therefore, using biodegradation products of food wastes would be an economical and sustainable approach. There is a rapidly growing literature, which indicates that the production of energy [37], biohydrogen [38,39], and PHA [40–43] are possible from the biodegradation of food wastes. The first study on PHA production from the organic fraction of municipal solid waste showed that production of around 33 g of PHA per 1 kg of fresh waste could be possible using VFA from acidification of the waste [34]. PHA content of the sludge can be achieved up to 40% by mixed cultures fed with fermented food waste [21], which is a significant constraint with mixed culture PHA production systems [44].

The present study attempted to investigate the optimization of PHA production using acclimated activated sludge, and VFA obtained from acidification of dairy wastewaters (DW) and food waste collected from a dining facility. To evaluate the effect of operational strategies on the selection of activated sludge with high PHA storage capacity, seed activated sludge was acclimated in lab-scale sequencing batch reactors (SBR) by applying feast-famine (FF) regime and anaerobic-aerobic-anoxic (AnAA) operation conditions fed with domestic wastewater only and with VFA supplemented.

2. Materials and methods

2.1. Set-up and operation of sequencing batch reactors

Enrichment of biomass with PHA store capacity was carried out in two lab-scale activated sludge systems consisted of SBRs with a working volume of 4 L operated parallel to offer FF (SBR-1) and anaerobic-aerobic-anoxic (AnAA) (SBR-2) growth conditions. Each reactor was initially seeded with excess activated sludge obtained from a municipal wastewater treatment plant, and fed with synthetic wastewater reflecting similar characteristics and COD fractions with domestic wastewater. The synthetic wastewater contained acetic acid, yeast extract, glucose, starch, and peptone described by Morgan-Sagastume et al. [17]. The PHA content of seed sludge was 0.9%.

SBR-1 operated with a 5 h reaction phase followed by 1 h of settling, decanting, and idle periods to provide a substrate gradient to compromise FF environment. Simultaneous feeding and aeration were initiated at the beginning of each cycle. The influent wastewater was fed into the reactor with total chemical oxygen demand (tCOD) concentration of around 500 mg COD/L during the operation period of SBR-1 with a flow-rate of 12 L/d. The lab-scale reactor was operated for more than 80 d in a constant temperature room maintained at 20°C ± 2°C under an organic loading rate of 1.5 g COD/L d and a sludge age of 3 and 6 d, where each operation accordingly named as FF3 and FF6.

The experimental data required for evaluating the PHA accumulation capability of AnAA growth conditions were gathered from SBR-2, which was continuously operated in an AnAA sequence with a short post-aerobic phase to improve sludge settleability. The reactor was operated in sequential cycles of 4 h:60 min of anaerobic reaction including fast feeding of 5 min with a flow rate of 15 L/d; 60 min of aeration; 45 min of anoxic; 15 min of aeration to improve setting properties of the sludge; 60 min of settling, decanting and idle. The tCOD concentration of the wastewater was around 400 mg COD/L. The reactor was operated for more than 80 d in a constant temperature room maintained at 20°C ± 2°C under an organic loading rate of 1.5 g COD/L d, which is identical to the SBR-1, and a sludge age of 8 (AnAA8) and 12 (AnAA12) d.

To determine the enhancement in PHA accumulation capability of the sludge with fermentative VFA supplement, an adequate volume of supernatant taken from fermentation vessels was added daily into synthetic wastewater. The corresponding COD concentration of fermentative total volatile fatty acid (tVFA) was kept constant as 100 mg/L for each SBR. Following the completion of FF6 and AnAA12, the operation conditions of both SBRs were kept identical. Supplementary fermentative VFA addition was started, and the runs were named as FF6$^+$ and AnAA12$^+$, where plus (+) corresponds to the addition of VFA.

2.2. Acidification experiments for VFA production

Acidification experiments were conducted in glass bottles with a total volume of 1 L and a working volume of almost 500 mL maintained at 37°C ± 1°C, where an adequate amount of DW or organic food waste (OFW) paste was fermented according to the selected substrate to inoculum (S/I). The total COD and total Kjeldahl nitrogen (TKN) concentrations of the original dairy wastewater were 14550 ± 355 mg/L and 700 ± 20 mg/L, respectively, which yield a COD/TKN ratio of 21. The average concentrations of 1 g of wet OFW were 343 ± 22 mg COD/L and 7.8 ± 0.8 mg TKN/L, which equates to a COD/TKN ratio of 44.

The inoculum biomass was obtained from an anaerobic digester of the food industry. The concentration of total suspended solids (TSS) and volatile suspended solids (VSS) in the inoculum were 357 and 73 g/L, respectively. The experiments were designed for S/I ratios of 1, 2.5, 5, and 10 g tCOD/g VSS. The wastewater volume and the amount of food waste were determined to ensure an initial tCOD mass of 7300 mg in each bottle, which corresponds to the selected tCOD concentration (14,600 mg/L) of dairy wastewater.

The simulated dairy wastewater was prepared by using commercial whole milk. tCOD concentration was initially characterized as 97 g COD per mL. The organic food waste containing chicken, vegetables, pasta, and beans was collected from the dining hall at Istanbul Technical University and then grounded to paste in a grinding machine. The bottles' content was purged with nitrogen gas to remove any residual oxygen after the addition of substrate and inoculum biomass, and then sealed tightly and shake once per day. All the fermentation experiments lasted for 6 d. During the tests, the VFA production was monitored by measuring acetic acid, propionic acid, valeric acid, butyric acid, isobutyric, isovaleric acid, caproic acid, and heptanoic acid. Total volatile fatty acid was calculated by converting each acid concentration to equivalent COD concentration ($tCOD_{VFA}$). The acidification yield was calculated and presented as g $tCOD_{VFA}$/g VSS and percentage of $tCOD_{VFA}$ in tCOD input.

For the PHA accumulation tests, the acidification experiments were later repeated as described above, utilizing the selected initial S/I ratio, in which maximum acidification yield was achieved. The acidified content of dairy wastewater and food waste was separated from the residue by centrifuging at 6,000 g for 5 min, immediately characterized and used for PHA accumulation tests as a VFA source.

2.3. Experimental design of batch tests for PHA accumulation

The PHA production potential of the fermented DW and OFW was studied using enriched activated sludge biomass under both aerobic and anaerobic batch conditions. The supernatant of acidified sources was used as a VFA source. Since the acidification efficiencies were varied from batch to batch, the actual characterization was used to estimate the production of PHA derived from VFA produced in the experiments. VFA consumption and PHA production were monitored during the batch experiments by collecting samples every 30–60 min intervals for 5 h. The characterization of batch experiments for PHA production using each substrate as a VFA source was represented in Table 1.

2.4. Analytical techniques

In this study, the substrate and biomass samples were characterized in terms of solids, COD, VFA, and PHA. COD measurements were accomplished by the ISO 6060 method [45]. The samples were filtered through Millipore membrane filters with a pore size of 0.45 mm for the soluble COD. The TSS and VSS analysis were performed as defined in Standard Methods [46]. PHA sampling and analysis were conducted according to the method proposed by Beun et al. [47]. The extraction procedure (addition of acid and 1,2-dichloroethane, and boiling for 2 h at 100°C) was followed after sample preparation (washing, freeze-drying, and weighing). VFA and extracted PHA analysis were conducted in Agilent 6890N gas chromatograph equipped with a flame ionization detector and two different columns. A capillary column (INNOWax 19095N-123) and an HP-FFAP capillary column (0.53 mm, 30 m, 1 μm) were used for PHA and VFA measurements, respectively. For PHA analysis, benzoic acid was used as an internal standard. A standard PHB/PHV (88%/12%) and caproic acid sodium salt solution were prepared and used for calibration. All samples were filtered through syringe filters with a 0.22 μm pore size before the measurement of VFAs.

The PHA content of the sludge was calculated as a percentage of VSS on cell dry weight (CDW). The PHA storage yield was calculated in mg COD_{PHA}/mg COD_{VFA} by dividing the amount of PHA produced by the total amount of VFA (in terms of COD) consumed during the batch test at maximum accumulation.

3. Results and discussion

3.1. Enrichment of PHA accumulating biomass

Enrichment of PHA accumulating mixed culture was carried out in two SBRs operated under FF regime at a sludge age of 3 and 6 d and AnAA conditions at sludge of 8 and 12 d. The PHA content of activated sludge in the SBRs was monitored until it was judged that the systems were under steady-state conditions during operation cycles, along with enrichment time.

In SBR operated under FF regime, the system sustained a suspended solids (SS) concentration of $1,540 \pm 145$ mg/L, $2,420 \pm 235$ mg/L, and $3,350 \pm 280$ mg/L with a VSS/SS ratio of 87%, 79% and 88% for FF3, FF6, and FF6+, respectively, at the steady-state. The maximum PHA contents of the sludge were obtained at 30 min of operation as 5.7%, 4.3%, and 2.8% at a sludge age of 3 d (FF3), 6 d (FF6), and 6 d with external VFA addition (FF6+), respectively. At the end of the cycle, the PHA content of the sludge was increased to 3.2% after 55 d operation of FF3, immediately decreased to 2.6% after 42 d operation of FF6, and unexpectedly not enhanced after the addition of external fermented VFA into wastewater (FF6+). The maximum PHA content of the biomass was achieved as 5.67%, 4.32%, and 12.80% at 30 min of operation for FF3, FF6, and FF6+, respectively. Therefore, the results revealed that the PHA content began to consume since there was no available carbon source in the mixed liquor after 30 min.

In SBR operated at AnAA conditions, suspended solids concentration were $4,600 \pm 250$ mg/L, $3,185 \pm 290$ mg/L and $3,830 \pm 215$ mg/L with a VSS/SS ratio of 83%, 78% and 86% for AnAA8, AnAA12 and AnAA12+, respectively, at the steady-state. The highest PHA content of the sludge has reached the value of 4.2% at 120 min for AnAA8, 3.7% at 60 min for AnAA12, and 12.4% at 60 min for AnAA12+. The PHA concentrations were started to decrease after depletion of the substrate, and PHA content was realized as 3.6%, 2.0%, and 10% at the end of the reaction period for AnAA8, AnAA12, and AnAA12+, respectively. The maximum PHA content of the biomass was achieved as 4.95, 3.74, and 12.36% at the end of the anaerobic phase for AnAA8, AnAA12, and AnAA12+, respectively.

The conventional parameters (solids, effluent COD, PO_4–P, and Nox–N) and PHA concentration were monitored during the cycle operations, and the results depicted in Fig. 1 for both SBRs. As shown from the figure, higher COD, phosphate, and nitrogen removal efficiencies were attained in the AnAA reactor compared to the complete aerobic reactor operated under the FF regime. Effluent oxidized nitrogen concentration was high at a sludge age of 6 d, and decreased from 12 to 7 mg/L with the addition of VFA into wastewater during FF operation, and always lower than 2 mg/L during AnAA operation, which indicating that removal of phosphate and nitrogen largely depend on sludge age and addition of VFA in all systems.

3.2. Acidification of dairy wastewater and food waste

In order to provide VFA for the PHA accumulation test, a series of primarily acidogenic fermentation tests were conducted using DW and OFW as carbon source and designed to determine optimal initial S/I ratio and sludge retention time yielding maximum VFA production.

All acidification tests were initiated with almost identical tCOD concentrations for comparative evaluation. As shown in Fig. 2, the tVFA profiles demonstrate similar trends for the substrate sources submitted to the acidification treatments and a little higher tVFA production for OFW than that of the tVFA produced from DW. The maximum tVFA concentrations on the day of 6 were obtained as 7,550 mg $tCOD_{VFA}$/L for DW and 6,800 mg $tCOD_{VFA}$/L for OFW. The results also indicated that the degradation of DW and OFW constituents was demonstrated slightly lower tVFA productions after 4 d. Considering that the initial concentration of tCOD was around 14,600 mg/L (7,300 mg tCOD/500 mL of test vessel), the resulting acidification efficiencies were reached to 31.1%, 47.5%, 49.1%, and 51.7% for DW, and 9.3%, 22.9%, 36.1% and 46.6% for OFW at applied S/I ratios of 1, 2.5, 5 and 10 at 6th day of the acidification. tVFA concentration was begun to decrease at low S/I ratios (at S/I ratio of 1 for DW, and S/I ratios of 1 and 2.5 for OFW) since all available carbon was depleted and presumably, VFA started to be consumed to produce methane after 4th day. The acidification yields obtained at an S/I ratio of 10 were 5.2 g COD/g VSS (51.7%) for DW and 3.7 g COD/g VSS (46.6%) for OFW at 4th d. Therefore, the selected conditions were determined as sludge retention time of 4 d and S/I ratio of 10 g COD/g VSS where higher acidification yield was achieved, and later applied for the following batch experiments to investigate PHA accumulation capability of enriched activated sludge when fed with acidified VFA attained from substrate sources.

3.3. PHA accumulation batch experiments

In the context of enhancement of PHA accumulation using fermented VFA obtained from acidification of DW and OFW, SBR operations have provided acclimated biomass to the following batch experiments. At the first stage, PHA accumulation potential of activated sludge taken from operations of FF3 and AnAA8 investigated for different food to microorganism ratios (tVFA/X), expressed as g $tCOD_{VFA}$/g VSS, and various conditions (anaerobic or aerobic) to determine successive test condition (aerobic or anaerobic) in terms of PHA accumulation. The characteristics of PHA accumulation tests, and the results of PHA content and storage yield values were given in Table 1. As shown in Table 1 and Fig. 3, the PHA content was affected significantly by the tVFA/X ratio and the test conditions, in which the significant alterations were observed for OFW. The activated sludge samples had PHA accumulation capability up to 67.34% of CDW under aerobic conditions and at a tVFA/X ratio of 1.57 g $tCOD_{VFA}$/g VSS when the fermented VFA source was OFW. In this batch, the maximum conversion yield from VFA to PHA was obtained as 0.687 g COD_{PHA}/g COD_{VFA}, comparable to those reported in the literature [34,48].

The results also show that the PHA content of the activated sludge associated with different tVFA/X ratios may significantly depend on the source of the fermented VFA, while the trend of PHA yield at different tVFA/X ratios was decreasing significantly for tVFA/X > 2 for both fermented VFA sources. As shown in Fig. 3, PHA accumulation under
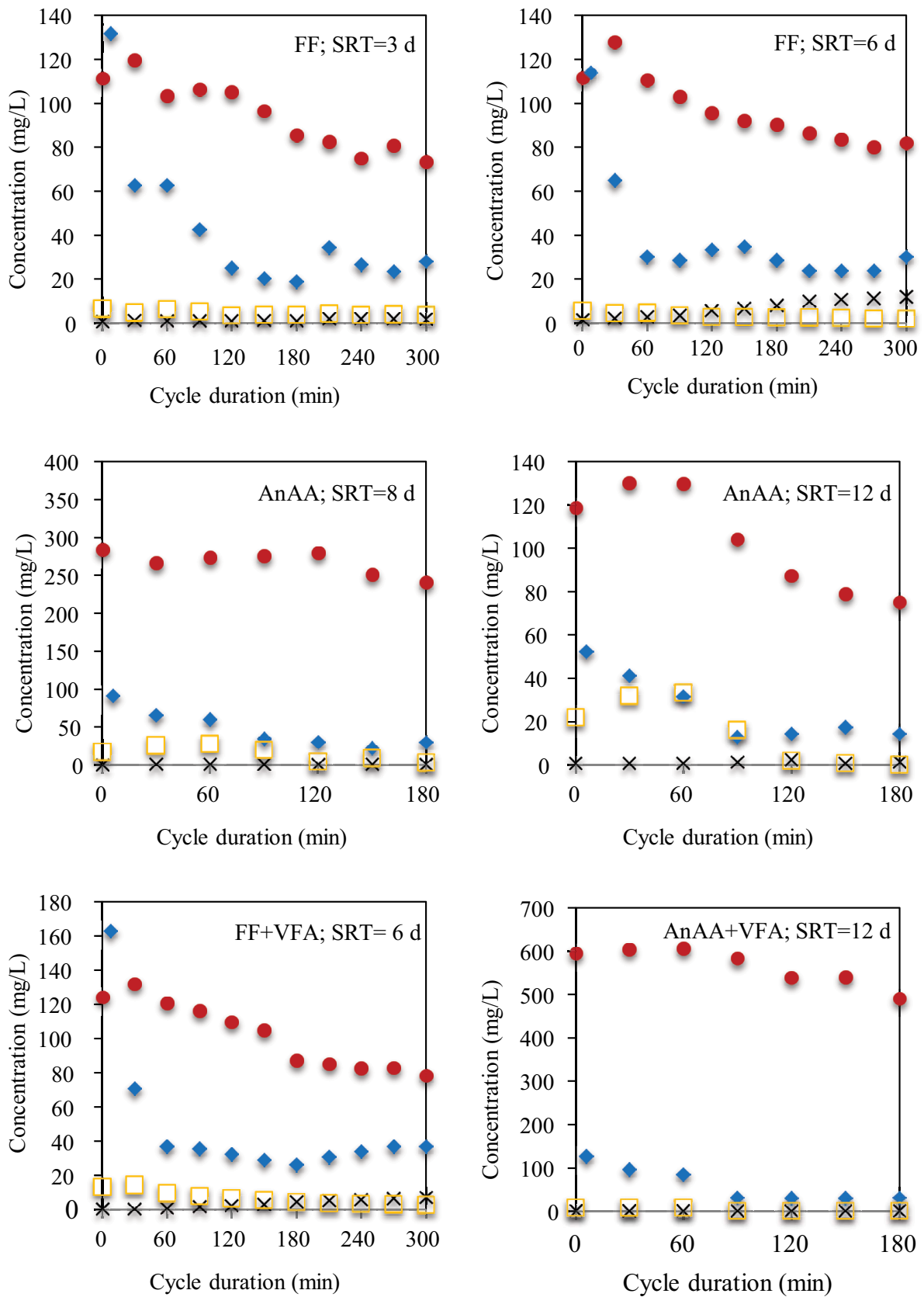
Fig. 1. ● PHA (as COD), ◆ COD, □ PO_4–P and × No_x–N concentration profiles of SBR cycle at steady-state conditions.
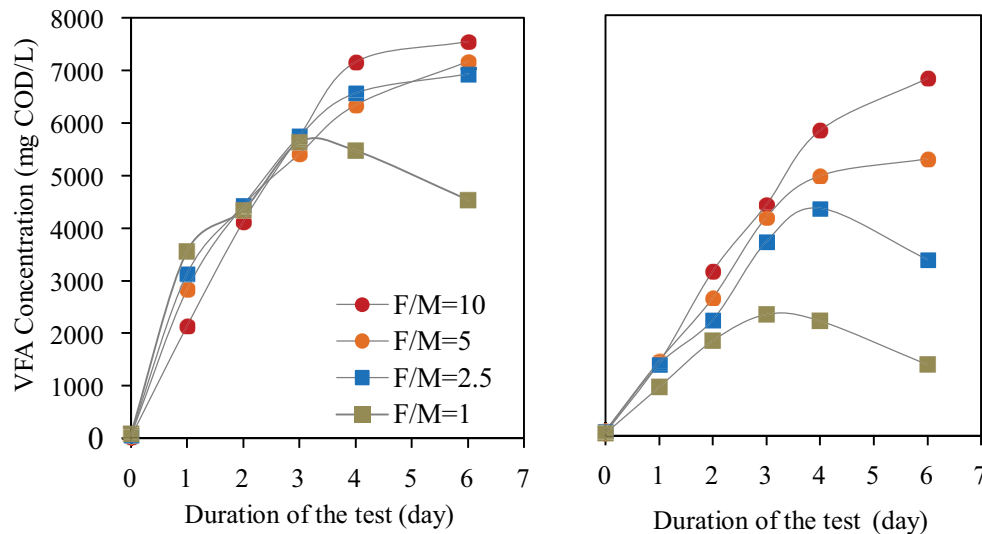
Fig. 2. tVFA profiles in acidification vessels for DW (a) and OFW (b) at different S/I ratios.

aerobic conditions attains the highest value at the tVFA/X ratio of 3 and 1.5, when fermented VFA obtained correspondingly from DW and OFW. Since there were almost identical or lower PHA accumulations during the anaerobic conditions compared to aerobic conditions, the test condition was preferred as aerobic for the following tests. It was apparent from these results that tVFA/X ratio is one of the essential parameters for PHA accumulation.

Then, the activated sludge samples from all FF6, FF6⁺, AnAA12, and AnAA12⁺ operations introduced into PHA accumulation tests only under aerobic conditions. The VFA was obtained from the supernatant of acidification vessels, and initial VFA concentrations were varied depending on the efficiency of the process, most likely because of changes in the composition of carbon source used during acidification of wastewater and waste.

Within 5 h of each test, complete VFA removal was not achieved. Thus, remaining VFA develops the claim that there would be a limitation by any following: low PHA accumulation capacity of acclimated sludge, VFA inhibition at high levels, and some of produced VFA constituents not suitable for PHA accumulation. The variations of PHA concentration were monitored during the tests and presented in Fig. 4. High concentrations of PHA were observed for acidified OFW, while lower PHA concentration was observed with acidified DW during the entire accumulation tests. Since the tVFA/X ratios applied to both VFA sources were almost in a limited range, it is proposed that the data yielded by this study provide convincing evidence that VFA from acidified OFW encourages PHA accumulation almost regardless of the origin of the sludge. The data gathered from tests also suggest that the activated sludge from SBR with the addition of external VFA into the feed was enhanced the PHA accumulation capacity of the sludge.

Fig. 5 shows that the fermentation products were dominated by acetic acid and isobutyric acid for both acidified VFA sources. Accordingly, a considerable proportion of the PHA consisted of PHB, as shown in Fig. 6. It should be

emphasized here that there might be other VFA constituents, which were not be measured in this study.

During the continuous operation of the acclimation reactor, a higher mass of PHA was gathered from PHA storage tests using the strategy used in this study, which was the introduction of permeate of acidified DW and OFW into domestic wastewater as an additional VFA source. Likely, as shown in Fig. 6, the higher PHA content was obtained from PHA accumulation batch tests, where the sludge originated from the SBR fed with additional VFA. On the other hand, however, the PHA content levels of the sludge conceived from these tests were still lower compared to the levels attained at lower sludge ages. Therefore, it was concluded that sludge age selection is of importance than feeding a continuous system with an additional VFA.

4. Conclusion

This study evaluated the influence of acidified VFA obtained from DW and OFW on PHA production using enriched activated sludge exposed to the FF regime and AnAA operation in an SBR. The data obtained from this study showed that PHA accumulation potential was relatively high using the FF regime, and sludge age would be particularly effective in selectively enriching PHA accumulating organisms. The PHA accumulation potential of acclimated activated sludge depends largely on the source of VFA constituents available for polymer storage rather than acclimation conditions.

The high resultant VFA concentrations produced in this study as consequences of acidification of selected wastes suggest that acidification of wastes appears as a promising approach for PHA accumulation purposes. The maximum acidification yields were obtained at the S/I ratio of 10 as 51.7% and 46.6% for DW and OFW at 4th d, respectively. As a result of PHA accumulation of acclimated sludge under aerobic conditions using VFA obtained from acidification of DW and OFW, the PHA contents increased up to 67.3%

Table 1
Characteristics of polyhydroxyalkanoates accumulation batch tests

Source of VFA	Regime[a]	Sludge age[a] (d)	Cond.[b]	VFA add.[c]	Trial	tVFA/X (g COD/g VSS)	PHA content (%)	PHA yield (g COD$_{PHA}$/g COD$_{VFA}$)
Dairy waste-water	FF	3			1	1.46	3.85	0.042
			An.		2	3.03	4.13	0.022
				No	3	4.2	3.08	0.012
					4	1.46	2.64	0.029
					5	3.03	4.47	0.024
		6	Ae.		6	4.2	4.16	0.016
					7	2.9	4.39	0.024
				Yes	8	2.31	3.01	0.021
	AnAA	8			9	2.17	3.67	0.027
			An.		10	3.62	2.87	0.013
				No	11	4.96	2.14	0.007
					12	2.17	3.67	0.027
					13	3.62	3.85	0.017
		12	Ae.		14	4.96	2.14	0.007
					15	4.02	3.36	0.013
				Yes	16	2.29	3.81	0.027
Organic food waste	FF	3			17	0.97	3.47	0.057
			An.		18	1.12	4.18	0.060
				No	19	1.57	1.36	0.014
					20	0.97	25.13	0.416
					21	1.12	44.27	0.631
		6	Ae.		22	1.57	67.34	0.687
					23	2.27	23.35	0.165
				Yes	24	1.73	32.22	0.298
	AnAA	8			25	1.04	8.11	0.124
			An.		26	1.36	8.27	0.097
				No	27	1.63	7.16	0.070
					28	1.04	25.68	0.394
					29	1.36	41.05	0.483
			Ae.		30	1.63	54.51	0.536
		12			31	3.14	19.95	0.102
				Yes	32	1.71	32.1	0.300

[a]Operational condition (FF: feast-famine; AnAA: anaerobic-aerobic-anoxic)/sludge age of the SBR, where the sludge used for the batch test was originated.
[b]Condition of the batch test (An: anaerobic; Ae: aerobic).
[c]Availability of additional feeding of fermented VFA into SBR, where the sludge used for the batch test was originated.

and 4.5% of CDW, respectively. It was apparent from the results of this study that the application of the FF regime and preferring short solid retention time (4 d) in the activated sludge system for the selection of PHA accumulating biomass, and tVFA/X ratio is one of the essential parameters for PHA accumulation stage.

Unfortunately, very low PHA content was attained using fermentation products of DW as a carbon source in this study, and this result is attributed to the high nitrogen content of the acidified supernatant, which presumably limited the PHA accumulation and organic matter is diverted to mostly growth instead of PHA synthesis. Pretreatment strategies for using fermented dairy wastewater should be further investigated. According to the results of this study, DW is still has a potential for PHA production, considering the generation of large amounts of wastewater from the dairy industry.

Accordingly, 173 kg of polymer from 1,000 m^3 of DW and 20.3 kg of polymer from 1 ton of the wet weight of OFW could be produced with the continuous operation of the proposed integrated system containing an activated sludge treatment plant and acidification reactor. This could be attributed to a significant profit when a large amount of DW and OFW considered. For the meaningfulness of this study, a specific economic evaluation should be further conducted and assessed.
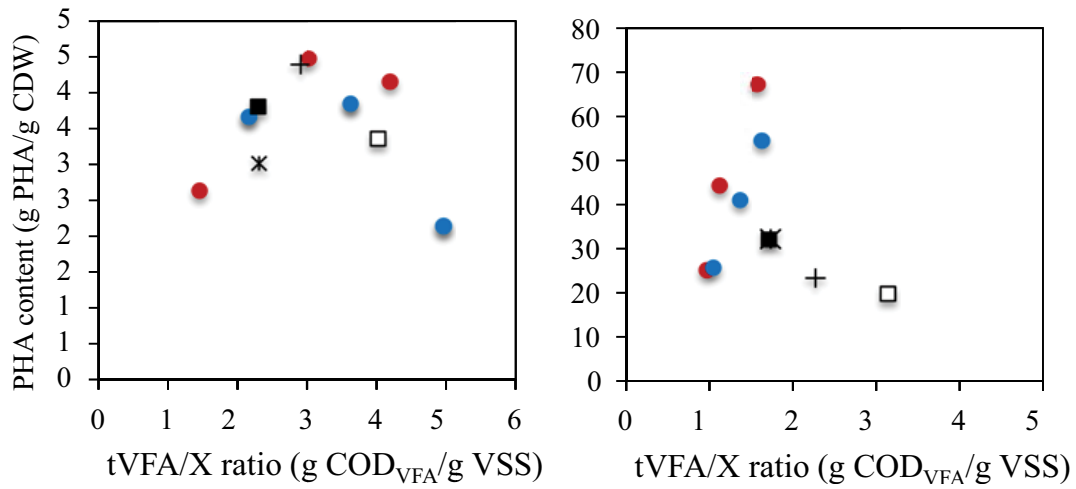
Fig. 3. PHA accumulation of activated sludge taken from SBR operations of ●FF3, +FF6, ×FF6⁺, ●AnAA8, □AnAA12 and ■AnAA12⁺ at different tVFA/X ratios using fermented VFA as a carbon source from DW (a) and OFW (b) under aerobic conditions.
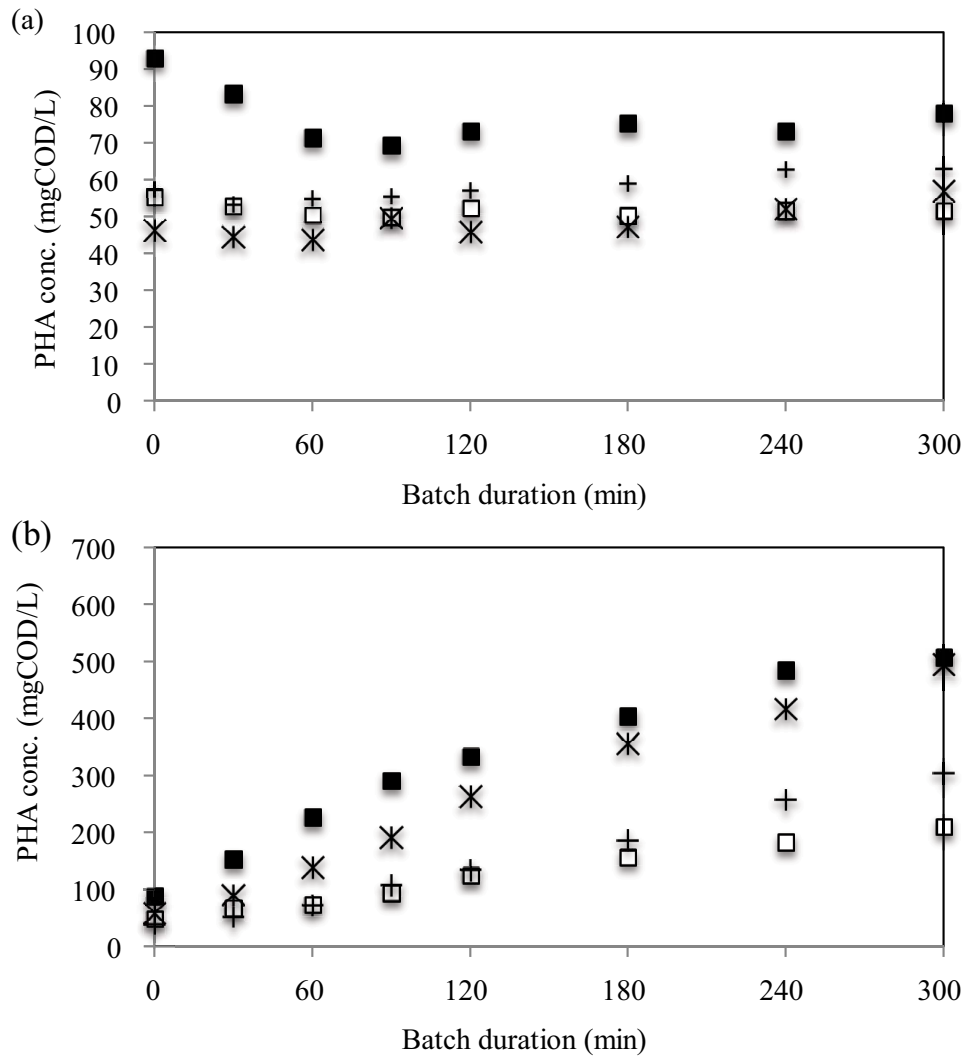

Fig. 4. PHA concentration profiles in PHA accumulation tests conducted with fermented VFA from (a) DW, and (b) OFW (+FF6, ●FF6⁺, □AnAA12 and ■AnAA12⁺).
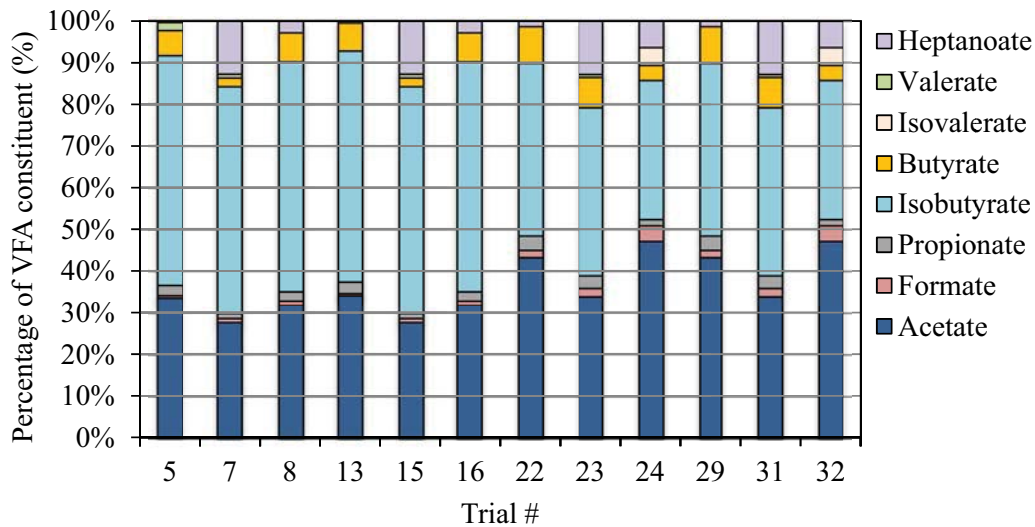
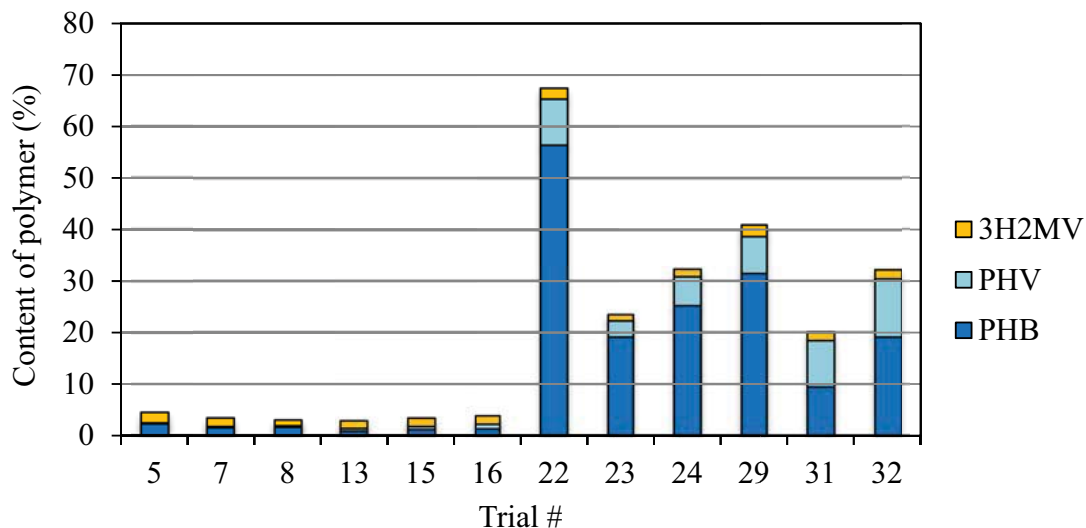Fig. 5. Composition of initial VFA used for PHA accumulations tests.

Fig. 6. Composition and percentage of CDW of PHA polymer at the end of the accumulation tests.

References

[1] R. Verma, K.S. Vinoda, M. Papireddy, A.N.S. Gowda, Toxic pollutants from plastic waste - a review, Procedia Environ. Sci., 35 (2016) 701–708.

[2] S.A. Ashter, Introduction to Bioplastics Engineering, William Andrew, 2016.[AQ1]

[3] L.S. Dilkes-Hoffman, J.L. Lane, T. Grant, S. Pratt, P.A. Lant, B. Laycock, Environmental impact of biodegradable food packaging when considering food waste, J. Cleaner Prod., 180 (2018) 325–334.

[4] T. Mekonnen, P. Mussone, H. Khalil, D. Bressler, Progress in bio-based plastics and plasticizing modifications, J. Mater. Chem. A, 1 (2013) 13379–13398.

[5] Z.A. Raza, S. Abid, I.M. Banat, Polyhydroxyalkanoates: characteristics, production, recent developments and applications, Int. Biodeterior. Biodegrad., 126 (2018) 45–56.

[6] H. Takabatake, H. Satoh, T. Mino, T. Matsuo, Recovery of biodegradable plastics from activated sludge process, Water Sci. Technol., 42 (2000) 351–356.

[7] E. Bugnicourt, P. Cinelli, A. Lazzeri, V. Alvarez, Polyhydroxyalkanoate (PHA): review of synthesis, characteristics, processing and potential applications in packaging, eXPRESS Polym. Lett., 8 (2014) 791–808.

[8] M.A. Hassan, L.-N. Yee, P.-L. Yee, H. Ariffin, A.R. Raha, Y. Shirai, Y., K. Sudesh, Sustainable production of polyhydroxyalkanoates from renewable oil-palm biomass, Biomass Bioenergy, 50 (2013) 1–9.

[9] J.E. Yang, S.Y. Choi, J.H. Shin, S.J. Park, S.Y. Lee, Microbial production of lactate-containing polyesters, Microb. Biotechnol., 6 (2013) 621–636.

[10] S. Yan, R.D. Bala Subramanian, R.D. Tyagi, R.Y. Surampalli, Chapter 6 – Bioplastics from Activated Sludge, R.D. Tyagi, R.Y. Surampalli, S. Yan, T.C. Zhang, C.M. Kao, B.N. Lohani,

Eds., Sustainable Sludge Management: Production of Value Added Products, American Society of Civil Engineers, 2009, pp. 123–145.

[11] P. Basnett, I. Roy, Microbial Production of Biodegradable Polymers and Their Role in Cardiac Stent Development, A. Méndez-Vilas, Eds., Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, Formatex Research Center, 2010, pp. 1405–1415.

[12] F. Morgan-Sagastume, S. Heimersson, G. Laera, A. Werker, M. Svanström, Techno-environmental assessment of integrating polyhydroxyalkanoate (PHA) production with services of municipal wastewater treatment, J. Cleaner Prod., 137 (2016) 1368–1381.

[13] L.S. Serafim, P.C. Lemos, M.G.E. Albuquerque, M.A.M. Reis, Strategies for PHA production by mixed cultures and renewable waste materials, Appl. Microbiol. Biotechnol., 81 (2008) 615–628.

[14] S. Chanprateep, Current trends in biodegradable poly-hydroxyalkanoates, J. Biosci. Bioeng., 110 (2010) 621–632.

[15] C. Hong, H. Hao, W. Haiyun, Process optimization for PHA production by activated sludge using response surface methodology, Biomass Bioenergy, 33 (2009) 721–727.

[16] A.S.M. Chua, H. Takabatake, H. Satoh, T. Mino, Production of polyhydroxyalkanoates (PHA) by activated sludge treating municipal wastewater: effect of pH, sludge retention time (SRT), and acetate concentration in influent, Water Res., 37 (2003) 3602–3611.

[17] F. Morgan-Sagastume, F. Valentino, M. Hjort, D. Cirne, L. Karabegovic, F. Gerardin, P. Johansson, A. Karlsson, P. Magnusson, T. Alexandersson, S. Bengtsson, M. Majone, A. Werker, Polyhydroxyalkanoate (PHA) production from sludge and municipal wastewater treatment, Water Sci. Technol., 69 (2014) 177–184.

[18] Y. Jiang, L. Marang, J. Tamis, M.C.M. van Loosdrecht, H. Dijkman, R. Kleerebezem, Waste to resource: converting paper mill wastewater to bioplastic, Water Res., 46 (2012) 5517–5530.

[19] L. Montano-Herrera, B. Laycock, A. Werker, S. Pratt, The evolution of polymer composition during PHA accumulation: the significance of reducing equivalents, Bioengineering, 4 (2017) 20, doi: 10.3390/bioengineering4010020.

[20] M.G.E. Albuquerque, C.A.V. Torres, M.A.M. Reis, Polyhydroxyalkanoate (PHA) production by a mixed microbial culture using sugar molasses: effect of the influent substrate concentration on culture selection, Water Res., 44 (2010) 3419–3433.

[21] M.V. Reddy, S.V. Mohan, Effect of substrate load and nutrients concentration on the polyhydroxyalkanoates (PHA) production using mixed consortia through wastewater treatment, Bioresour. Technol., 114 (2012) 573–582.

[22] W.S. Lee, A.S.M. Chua, H.K. Yeoh, G.C. Ngoh, A review of the production and applications of waste-derived volatile fatty acids, Chem. Eng. J., 235 (2014) 83–99.

[23] A.A. Bazyar Lakeh, A. Azizi, E. Hosseini Koupaie, V. Bek-muradov, H. Hafez, E. Elbeshbishy, A comprehensive study for characteristics, acidogenic fermentation, and anaerobic digestion of source separated organics, J. Cleaner Prod., 228 (2019) 73–85.

[24] Y. Jiang, L. Marang, R. Kleerebezem, G. Muyzer, M.C.M. van Loosdrecht, Polyhydroxybutyrate production from lactate using a mixed microbial culture, Biotechnol. Bioeng., 108 (2011) 2022–2035.

[25] S. Bengtsson, A. Werker, M. Christensson, T. Welander, Production of polyhydroxyalkanoates by activated sludge treating a paper mill wastewater, Bioresour. Technol., 99 (2008) 509–516.

[26] A. Banerjee, P. Elefsiniotis, D. Tuhtar, Effect of HRT and temperature on the acidogenesis of municipal primary sludge and industrial wastewater, Water Sci. Technol., 38 (1998) 417–423.

[27] M.B. Salerno, H.-S. Lee, P. Parameswaran, B.E. Rittmann, Using a pulsed electric field as a pretreatment for improved biosolids digestion and methanogenesis, Water Environ. Res., 81 (2009) 831–839.

[28] B.E. Rittmann, P.L. McCarthy, Environmental Biotechnology: Principles and Applications, Tata McGraw Hill Education Private Limited, 2012.

[29] H.G. Yu, H.H. Fang, Acidogenesis of dairy wastewater at various pH levels, Water Sci. Technol., 45 (2002) 201–206.

[30] K. Wang, J. Yin, D. Shen, N. Li, Anaerobic digestion of food waste for volatile fatty acids (VFAs) production with different types of inoculum: effect of pH, Bioresour. Technol., 161 (2014) 395–401.

[31] T.J. Britz, C. van Schalkwyk, Y.-T. Hung, Treatment of Dairy Processing Wastewaters, Taylor & Francis Group, LLC, 2006.

[32] F. Carvalho, A.R. Prazeres, J. Rivas, Cheese whey wastewater: characterization and treatment, Sci. Total Environ., 445–446 (2013) 385–396.

[33] R.H. Zhang, H.M. El-Mashad, K. Hartman, F.Y. Wang, G.Q. Liu, C. Choate, P. Gamble, Characterization of food waste as feedstock for anaerobic digestion, Bioresour. Technol., 98 (2007) 929–935.

[34] B. Colombo, F. Favini, B. Scaglia, T.P. Sciarria, G. D'Imporzano, M. Pognani, A. Alekseeva, G. Eisele, C. Cosentino, F. Adani, Enhanced polyhydroxyalkanoate (PHA) production from the organic fraction of municipal solid waste by using mixed microbial culture, Biotechnol. Biofuels, 10 (2017) 201, doi: 10.1186/s13068-017-0888-8.

[35] B.K. Adhikari, S. Barrington, J. Martinez, S. King, Charac-terization of food waste and bulking agents for composting, Waste Manage., 28 (2008) 795–804.

[36] H. Fisgativa, A. Tremier, P. Dabert, Characterizing the variability of food waste quality: a need for efficient valorisation through anaerobic digestion, Waste Manage., 50 (2016) 264–274.

[37] C.M. Braguglia, A. Gallipoli, A. Gianico, P. Pagliaccia, Anaerobic bioconversion of food waste into energy: a critical review, Bioresour. Technol., 248 (2018) 37–56.

[38] Y.-H. Lin, H.-X. Zheng, M.-L. Juan, Biohydrogen production using waste activated sludge as a substrate from fructose-processing wastewater treatment, Process Saf. Environ. Prot., 90 (2012) 221–230.

[39] S.V. Mohan, L. Agarwal, G. Mohanakrishna, S. Srikanth, A. Kapley, H.J. Purohit, P.N. Sarma, Firmicutes with iron dependent hydrogenase drive hydrogen production in anae-robic bioreactor using distillery wastewater, Int. J. Hydrogen Energy, 36 (2011) 8234–8242.

[40] M. Koller, L. Maršálek, M.M. de Sousa Dias, G. Braunegg, Producing microbial polyhydroxyalkanoate (PHA) bio-polyesters in a sustainable manner, New Biotechnol., 37 (2017) 24–38.

[41] M. Koller, A. Salerno, A. Muhr, A. Reiterer, G. Braunegg, Polyhydroxyalkanoates: biodegradable polymers and plastics from renewable resources, Mater. Tehnol., 47 (2013) 5–12.

[42] M.M. Zhang, H.Y. Wu, H. Chen, Coupling of poly-hydroxyalkanoate production with volatile fatty acid from food wastes and excess sludge, Process Saf. Environ. Prot., 92 (2014) 171–178.

[43] C. Nielsen, A. Rahman, A.U. Rehman, M.K. Walsh, C.D. Miller, Food waste conversion to microbial polyhydroxyalkanoates, Microb. Biotechnol., 10 (2017) 1338–1352.

[44] K. Amulya, M.V. Reddy, M.V. Rohit, S.V. Mohan, Wastewater as renewable feedstock for bioplastics production: understanding the role of reactor microenvironment and system pH, J. Cleaner Prod., 112 (2016) 4618–4627.

[45] ISO 6060, Water Quality-Determination of the Chemical Oxygen Demand, International Organization for Standardization, Switzerland, Geneva, 1986.

[46] APHA/AWWA/WEF, Standard Methods for the Examination of Water and Wastewater, 21st ed., Vol. 21, American Public Health Association, Washington, D.C., 2005.

[47] J.J. Beun, F. Paletta, M.C.M. Van Loosdrecht, J.J. Heijnen, Stoichiometry and kinetics of poly-β-hydroxybutyrate metabolism in aerobic, slow growing, activated sludge cultures, Biotechnol. Bioeng., 67 (2000) 379–389.

[48] B. Colombo, T.P. Sciarria, M. Reis, B. Scaglia, F. Adani, Polyhydroxyalkanoates (PHAs) production from fermented cheese whey by using a mixed microbial culture, Bioresour. Technol., 218 (2016) 692–699.