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Pilot-Scale Polyhydroxyalkanoate Production from Paper Mill Wastewater: Process Characteristics and Identification of Bottlenecks for Full-Scale Implementation

Jelmer Tamis[1]; Michel Mulders[2]; Henk Dijkman[3]; René Rozendal[4]; Mark. C. M. van Loosdrecht[5]; and Robbert Kleerebezem[6]

Abstract: In this study, the suitability of paper industry wastewater for production of polyhydroxyalkanoate (PHA) was investigated in a pilot reactor in an industrial setting. The pilot plant was designed as a three-step process comprising (1) anaerobic fermentation for maximization of the volatile fatty acid (VFA) concentration, (2) enrichment of PHA-producing biomass, and (3) accumulation for maximization of the PHA content of the biomass. After fermentation, the paper mill process water contained a VFA fraction of 78% on a chemical oxygen demand (COD) basis. The length of the feast phase in the enrichment process stabilized at 45 min $\pm$ 4 min after 18 days of operation. At the end of the feast phase all VFA was consumed and the PHA content of the volatile suspended solids (VSS) was 0.50 g PHA/g VSS $\pm$ 0.05 g PHA/g VSS. The acquired microbial community was dominated by *Plasticicumulans acidivorans*, a PHA-producing microorganism previously found to dominate VFA-fed laboratory reactors. The maximum PHA content achieved after accumulation was 0.70 to 0.80 g PHA/g VSS. An overall PHA yield of 34% on a COD basis was achieved. Improving the VFA fraction in the product spectrum of the fermentation and minimization of acid and base consumption for pH control were identified as major bottlenecks. **DOI: 10.1061/(ASCE)EE.1943-7870.0001444.**

Introduction

In this paper we investigate waste-based polyhydroxyalkanoate (PHA) production, an innovative technology aiming for economic conversion of organic waste into more valuable products. The technology is based on microorganisms that recover organic compounds in wastewater as PHA polyesters for potential application, e.g., as a component for biodegradable plastics or as a building block for the chemical industry (Chen 2009; Kleerebezem and van Loosdrecht 2007; Reis et al. 2003). In wastewater-based biological processes, sterilization of the substrate and equipment is often not feasible, and it is therefore implausible to keep unwanted microbial species from entering the system. To address this issue, a desired trait in a wastewater-based biological process without medium and equipment sterilization can be achieved by implementation of a selective environment that provides a competitive advantage to species with a desired functionality. Previous research has shown that pulsewise dosing of volatile fatty acids (VFAs) provides a competitive advantage for microorganisms that store PHA and outgrow other microorganisms when external substrate is absent (Bengtsson et al. 2008; Beun et al. 2002; Dionisi et al. 2007; Johnson et al. 2009; Serafim et al. 2008). The development of such *open* microbial culture systems for the production of PHA from organic waste streams has been investigated at the pilot scale for municipal and food industry wastewaters (Anterrieu et al. 2014; Chakravarty et al. 2010; Morgan-Sagastume et al. 2014; Tamis et al. 2014a). These pilot studies have reported in all cases that the used microbial cultures were able to accumulate PHA in their cells, resulting in a biomass with PHA contents varying from 30 to 70% on a dry mass basis. Furthermore, it was observed that the PHA content and yield in pilot experiments were generally lower than those achieved in laboratory-scale studies with synthetic substrates (up to 90% on a dry mass basis) (Jiang et al. 2011c; Johnson et al. 2009; Marang et al. 2013). Until now, the highest cellular PHA contents in both pilot and laboratory experiments have been achieved with cultures dominated by the gamma-proteobacterial species *Plasticicumulans acidivorans*, which is apparently specialized in storing large quantities of PHA as carbon and energy storage (Jiang et al. 2010; Johnson et al. 2009; Tamis et al. 2014a).

In this study, the feasibility of producing PHA from wastewater at a paper mill was investigated using a pilot reactor system installed at the ESKA Graphic Board factory in Hoogezand, the Netherlands. Earlier laboratory-scale studies have already investigated this type of process water as potential feedstock for PHA production (Bengtsson et al. 2008; Jiang et al. 2012). The objective of this study was to investigate if there are important differences between laboratory-scale and pilot-scale experiments for identification of the most important bottlenecks for full-scale implementation. The hypothesis was that PHA production is feasible and robust in terms of product purity and yield under industrially relevant process conditions. Differences between pilot and laboratory experiments included operation without the use of expensive additives to prevent nitrification (i.e., allylthiourea) and operation with

[1]Process Specialist, Paques BV, Tjalke de Boerstritte 24, 8561 EL, Balk, Netherlands (corresponding author). ORCID: https://orcid.org/0000-0003-2001-7769. Email: j.tamis@tudelft.nl

[2]Ph.D. Candidate, Dept. of Biotechnology, Delft Univ. of Technology, Van der Maasweg 9, 2629 HZ, Delft, Netherlands.

[3]Senior Process Specialist, Paques BV, Tjalke de Boerstritte 24, 8561 EL, Balk, Netherlands.

[4]Chief Technology Officer, Paques BV, Tjalke de Boerstritte 24, 8561 EL, Balk, Netherlands.

[5]Professor, Dept. of Biotechnology, Delft Univ. of Technology, Van der Maasweg 9, 2629 HZ, Delft, Netherlands.

[6]Assistant Professor, Dept. of Biotechnology, Delft Univ. of Technology, Van der Maasweg 9, 2629 HZ, Delft, Netherlands.

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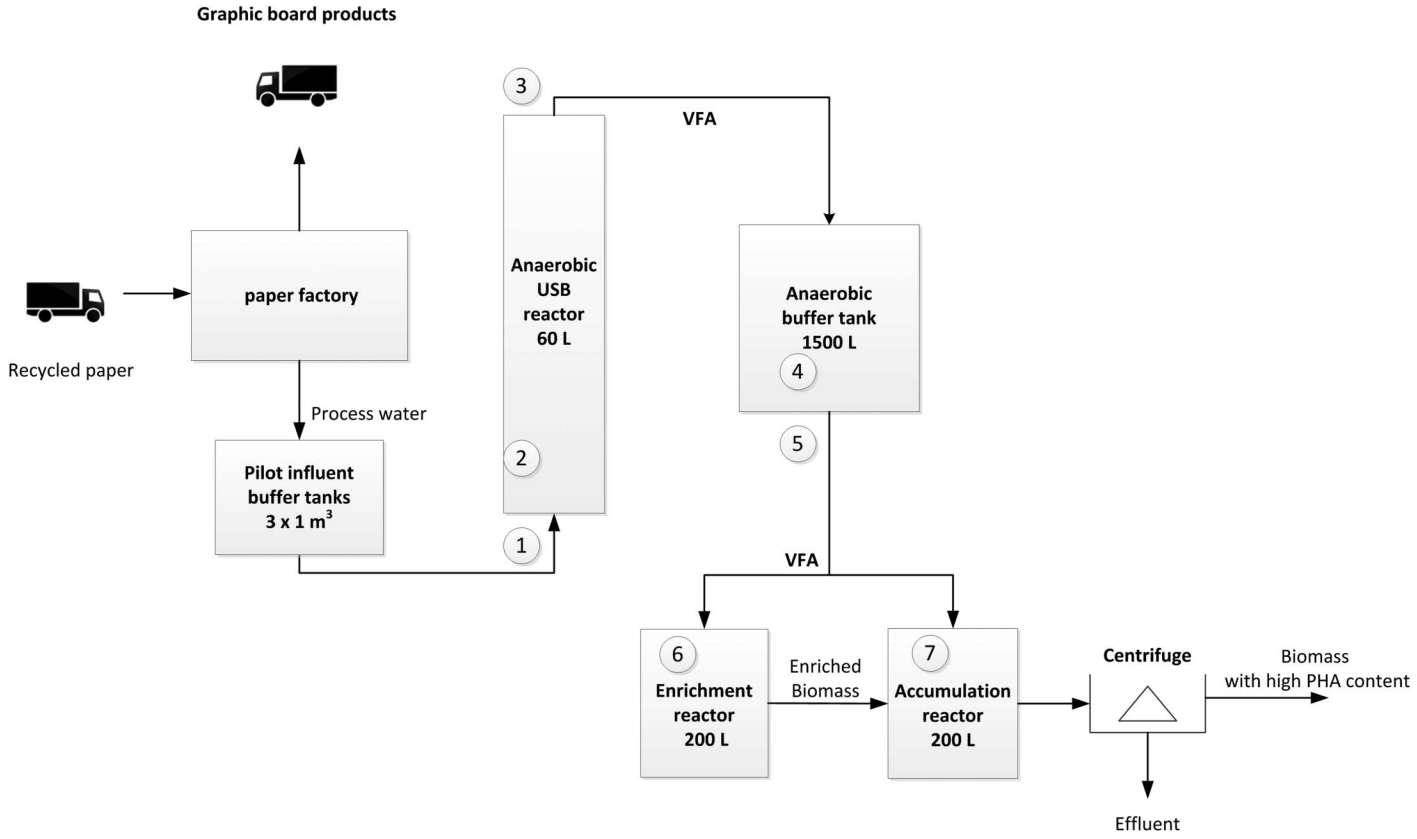

Fig. 1. Pilot system for PHA production from paper factory process water. The numbers indicate sample points. The unit operations in Sample Points 1–5 are related to anaerobic fermentation. Sample Point 6 is in the enrichment reactor and Sample Point 7 in the accumulation reactor.

less strict pH and temperature control and fluctuations in substrate concentrations occurring under industrial conditions.

Materials and Methods

A period of 42 days (July 28, 2015, until September 8, 2015) was investigated during which a system was operated based on feast-famine conditions similar to laboratory-scale systems in the literature (Johnson et al. 2009). The pilot plant was designed as a three-step process comprising (1) anaerobic fermentation for maximization of VFA concentrations, the preferred substrate for the subsequent steps; (2) enrichment for the production of biomass with superior PHA storage capacity; and (3) accumulation for the maximization of the PHA content of the biomass (Fig. 1).

Process Influent and Pilot-Scale Anaerobic Fermentation

The influent process water was diluted four times with water in order to mimic full-scale conditions, where the chemical oxygen demand (COD) concentations would be lower due to the presence of a wastewater treatment unit. In a full-scale situation, clarified process effluent instead of tap water would be used for dilution.

Maximization of the VFA concentrations for feeding the enrichment and accumulation reactors was pursued by anaerobic fermentation. To this end, an upflow anaerobic sludge blanket (UASB)–type reactor was operated similar to a laboratory-scale system reported in an earlier study (Tamis et al. 2015). The reactor had a liquid volume of 60 L and was operated at 30 ± 2°C; the pH was maintained at 5.0 ± 0.1 by controlled addition of 1 M NaOH. The UASB reactor was not inoculated; instead anaerobic bacteria originating from the wastewater were allowed to establish themselves in the reactor. The hydraulic retention time (HRT) of the reactor was around 3 h. The solid retention time (SRT) was not strictly controlled, but significant biomass retention was achieved and well-settling granular biomass was formed. Manual sludge removal was applied when the sludge bed exceeded 80% of the reactor volume. In order to stimulate growth of (VFA-producing) bacteria, a nutrient solution was dosed. The amount of nutrients dosed was adjusted to maintain N-limiting conditions (favorable for use in the accumulation step, later in the process) and the target COD:N mass ratio was around 200:1. The nutrient solution contained 3 M urea-N, 0.3 M phosphate, 0.3 M $MgSO_4$, 0.2 M K_2SO_4, 64 mM $FeCl_3$, 3 mM $ZnSO_4$, 2.7 mM H_3BO_3, 2.1 mM $NiCl_2$, 1.5 mM $CoSO_4$, 0.6 mM $CuSO_4$, and 0.8 mM Na_2MoO_4.

The effluent of the UASB reactor was stored in an anaerobic buffer vessel with a liquid volume of 1,500 L, a temperature controlled at 35 ± 5°C (5° warmer than the enrichment reactor to prevent cooling down of the enrichment reactor due to substrate dosing), and a pH controlled at 5.0 ± 0.1 by addition of 1 M NaOH. The HRT of 3 days was a resultant of the influent flow (480 L/day), which should be well above the amount of substrate required for the subsequent enrichment (170 L/day) and accumulation reactors (about 100–300 L per batch depending on the type of experiment). The SRT was not strictly controlled, i.e., there was no manual removal of sludge except for sludge concentrations in the reactor effluent, but since the buffer vessel was essentially not mixed, biomass retention was possible and the SRT was significantly longer than the 3-day HRT. The rationale behind the applied SRT in both anaerobic reactors was that acidogenic and acetogenic microorganisms should

have the chance to maintain themselves in the system, while acetoclastic methanogens should not. In order to check whether there was no excessive loss of soluble COD through methane production, influent- and effluent-soluble COD concentration were checked on a daily basis.

Pilot-Scale Enrichment Reactor

An aerobic bubble column reactor (height 2 m, diameter 0.4 m) was used for the enrichment and production of a biomass with superior PHA-storing capabilities. The reactor had a liquid volume of 170 L and was inoculated with activated sludge from a wastewater treatment plant (Dokhaven, Rotterdam, Netherlands). The reactor was aerated at 200 L/min, supplied through a fine bubble diffuser, to achieve mixing and to supply oxygen throughout the complete cycle. The reactor was temperature controlled at 30 ± 2°C using a warm water heating jacket. Controlled amounts of 1 M HCl and 1 M NaOH were pumped into the reactor to maintain the pH between 6.6 and 7.2.

In order to create an environment that provided a competitive growth advantage to PHA-producing bacteria, a feast-famine strategy was pursued based on the report by Johnson et al. (2009). The reactor was operated as a sequencing batch reactor (SBR) with a cycle length of 12 h and a solid and liquid retention time of 24 h. The operational cycle consisted of three phases:

1. Feed phase: During the feed phase, 85 L of fermented process water was dosed from the buffer vessel using a pH-controlled pump. The pump was programmed so that it only dosed the acidic substrate as long as the pH was above 6.6. and stopped dosing when the pH in the reactor went below 6.6. The substrate pump time was 11 min.
2. Nutrient dosing phase: Nutrients were dosed directly after the substrate dosage; the amount was based on daily measurement of the ammonium concentration at the end of the cycle. In this way, the concentration of ammonium could be maintained between 10 and 30 mgN/L at the end of the cycle. The resulting COD:N mass ratio that was fed to the reactor was approximately 30:1. Based on the composition of the nutrient medium (3 M urea-N, 0.3 M phosphate, 0.3 M $MgSO_4$, 0.2 M K_2SO_4, 64 mM $FeCl_3$, 3 mM $ZnSO_4$, 2.7 mM H_3BO_3, 2.1 mM $NiCl_2$, 1.5 mM $CoSO_4$, 0.6 mM $CuSO_4$, and 0.8 mM Na_2MoO_4), all nutrients were assumed to be in excess as compared to nitrogen. The nutrient dosing time was around 1 h.
3. Reaction phase: After all the substrate was dosed, the reactor was operated aerobically for a relatively long period during which no substrate was provided (i.e., around 11 h).
4. Effluent phase: At the end of the cycle, half of the aerated reactor content (85 L) was discharged as effluent or used for the subsequent accumulation step. The pump time of this phase was around 10 min and the total cycle time was 12 h.

Pilot-Scale Accumulation Reactor

To maximize the PHA content in the cells, a fed-batch reactor with a working volume 200 L was operated. The procedure was as follows: (1) the effluent from the enrichment reactor (85 L) containing the biomass was pumped into the accumulation reactor, (2) residual nitrogen was depleted by dosing a small pulse of substrate followed by a short famine period, (3) the depletion of ammonium was verified, and (4) the accumulation was initiated by dosing fermented process water from the buffer vessel using a pH-regulated pump, programmed so that substrate was only dosed when the pH in the reactor was above 6.6. The reactor was aerated through a fine bubble diffuser with a constant air flow of 200 L/min and the temperature was maintained at 30 ± 2°C. At the end of the accumulation, the biomass could be harvested using a pilot-scale centrifuge, processing a flow of 200 L/h of reactor broth at 3,000g.

Sampling and Analytical Methods

Filtered [0.45-μm pore size, polyvinylidene fluoride (PVDF) membrane, Millipore, Carraig Thuathail, Ireland] and unfiltered samples were collected to evaluate soluble and total COD using a spectrophotometric method provided by Hach Lange (Manchester, United Kingdom). Additional filtered samples were used for analysis of VFAs using high-performance liquid chromatography (HPLC) [Bio-Rad Aminex HPX-87H column, (Veenendaal, Netherlands) Waters 2489 Ultraviolet/Infrared (UV/RI) detector (Milford, Massachusetts), 1.5 mM H_3PO_4 mobile phase with a flow rate of 0.6 mL/min and a temperature of 60°C] and alcohols using gas chromatography (GC) [HP-INNOWax column (Agilent, Santa Clara, California) with a flame ionization detector (FID) detector, helium as carrier gas, and injector and detector and column temperatures of 200, 250, and 60°C, respectively]. Total and volatile suspended solid (TSS and VSS) concentration and the sludge volume index (SVI_{30}) were analyzed by standard methods (Clesceri et al. 1999). The PHA content of the biomass was evaluated using the method described by Johnson et al. (2009): weighed biomass samples and standards [pure poly-3-hydroxybutyric acid-co-3-hydroxyvaleric acid with a hydroxyvaleric (HV) content of 12 mol% as external standard; and benzoic acid in 1-propanol as the internal standard] were heated at 100°C for 2 h in a mixture of concentrated HCl, 1-propanol, and dichlorethane (volume ratios 1:4:5). The propylesters in the organic phase were analyzed by GC [Agilent Technologies Model 6890N (Santa Clara, California) equipped with an HP-INNOWax polyethylene glycol column with 60-m length, 250-μm inner diameter, and 0.15 μm film]. Ammonium was measured using a spectrophotometric method provided by Hach Lange. Nitrite and nitrate concentrations were analyzed using a colorimetric method (Merckoquant, Merck, Kenilworth, New Jersey).

Microbial Community Structure Analysis

The composition of the microbial community was analyzed by microscopic inspection of fluorescent in situ hybridization (FISH) slides with a UCB823 probe mixture for identification of *P. acidivorans* and a EUB338 probe mixture that binds with most types of eubacterial species. Additionally, light microscopy was used to evaluate if there were any cells that did not bind with either probe. The aim of the analysis was to identify the fraction of *P. acidivorans* cells compared with the total population (all other cells; cells not binding with UCB823 were defined as a side population in this study). No further in-depth analysis was performed as to the nature of the side population.

Data Analysis

The total VFA concentration was defined in this study as the sum of the individual COD concentrations of acetate, propionate, butyrate, and valerate. Soluble COD that was not identified as either VFA or ethanol was termed other soluble COD (other sCOD) throughout this paper. The solid COD was defined as the COD concentration of an unfiltered sample minus the COD concentration of a filtered sample. Biomass concentrations were calculated by subtracting the weight of storage compounds from the weight of volatile suspended solids. The length of the feast phase was used as an indicator for performance of the enrichment and process stability (Jiang et al. 2011b) and was identified using the dissolved oxygen (DO) profile. Characteristic process parameters were identified by calibrating a process model to the experimental data. The model was based

Table 1. Characteristic rates (q and k) and yields (Y) derived from cyle experiment in the enrichment reactor compared with other reactors operated at the same temperature (30°C)

Characteristic	This study	Mars pilot study[a]	Laboratory studies (acetate)[b]	Unit
q^{max}_{VFA}	7.8	2.8	4.1–5.6	g COD/g biomass/h
$q^{max}_{BCOD(nonVFA)}$	0.36	0.50	N/A	g COD/g biomass/h
k_{PHA}	0.21	0.21	N/A[c]	g $PHA^{(1/3)}$/g $biomass^{(1/3)}$/h
$Y_{PHA/VFA}$	0.40	0.37	0.40–0.45	g PHA/g COD
$Y_{X/PHA}$	0.75	0.82[d]	0.93	g biomass/g PHA
$Y_{X/VFA}$	0.30	0.30	0.34–0.35	g biomass/g COD

[a]Pilot study using effluent for the Mars candy bar factory as substrate (Tamis et al. 2014a).
[b]Johnson et al. (2009), Jiang et al. (2010, 2011a, b), and Marang et al. (2013).
[c]Difference in kinetic expressions used in model, making comparative analysis unfeasible.
[d]Calculated using the reported yield of 0.49 g biomass/g COD and a COD content of PHA of 1.67 g COD/g PHA.

on earlier publications (Tamis et al. 2014b) and is provided in the Supplemental Data. The aim of the model was to identify the following characteristic process variables (Table 1):

- Kinetic parameters: the maximum substrate uptake rate for VFA (q^{max}_{VFA}) and non-VFA COD ($q^{max}_{BCOD(nonVFA)}$) and the PHA degradation rate constant (k_{PHA}); and
- Stoichiometric parameters: the yield of PHA on VFA ($Y_{PHA/VFA}$), the yield of biomass on PHA ($Y_{X/PHA}$), and the yield of direct growth of biomass on COD ($Y_{PHA/BCOD}$).

Results

Influent Characterization and Fermentation

The influent process water had a pH of around 7 and a temperature of around 30°C, and was diluted four times with tap water before entering the pilot system resulting in an influent COD concentration of 6.9 ± 0.4 g COD/L (average $\pm$ standard deviation over the data set; $n = 42$). The dilution was chosen to mimic the COD concentration that would be achieved upon installation of a wastewater treatment unit in the process water loop. There were no significant concentrations of solids in the influent, i.e., less than 1% of the total COD. The VFA fraction in the influent process water was 0.28 ± 0.06 g COD/g COD and the alcohol fraction was not significant, i.e., <0.01 g COD/g COD. Since the measurement of VFA and alcohols could not explain the total concentration of COD measured using the spectrophototric method provided by Hach Lange, it was assumed that other organic compounds were present in the process water that could not be identified by the GC and HPLC methods used in this study. The soluble nitrogen concentration in the influent were generally low as compared to COD: total nitrogen was 18 ± 4 mg/L comprising <2 mg/L ammonium, <1 mg/L nitrite, <1 mg/L nitrate, and 16 mg/L organic nitrogen compounds that were not identified.

The UASB reactor was fed with the process water as influent and within a week a well-settling biomass formed in the reactor. Manual sludge removal resulted in an SRT of around 4 ± 1 days, much longer than the HRT of 3 h, thus favoring biomass with good settling properties. The soluble COD concentrations decreased on average 11% during the fermentation, an amount that corresponded well with COD contained in the produced biomass, suggesting that there was no major COD loss due to hydrogen or methane production (Fig. 2).

The influent contained relatively large quantities of lactate, which were almost completetly depleted in the subsequent fermentation steps and a significant net production of VFA was observed in the UASB reactor, with VFA fractions increasing from 0.28 to 0.72 g COD/g COD on average. After the fermentation in the UASB reactor there was a limited extent of further fermentation in the anaerobic buffer vessel, with an increase of the VFA fraction from 0.72 to 0.78 g COD/g COD on average. The fraction of uneven chain length VFA (propionate + valerate)/(acetate + propionate + butyrate + valerate) after fermentation was evaluated

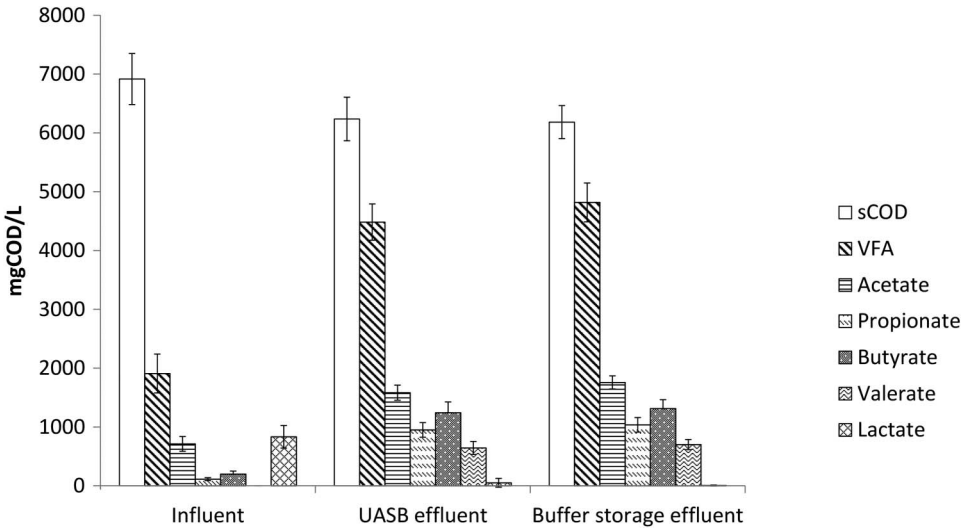

Fig. 2. Composition of the VFA fraction in the influent and the fermented process water. Error bars represent the standard deviation over the data set ($n = 42$).

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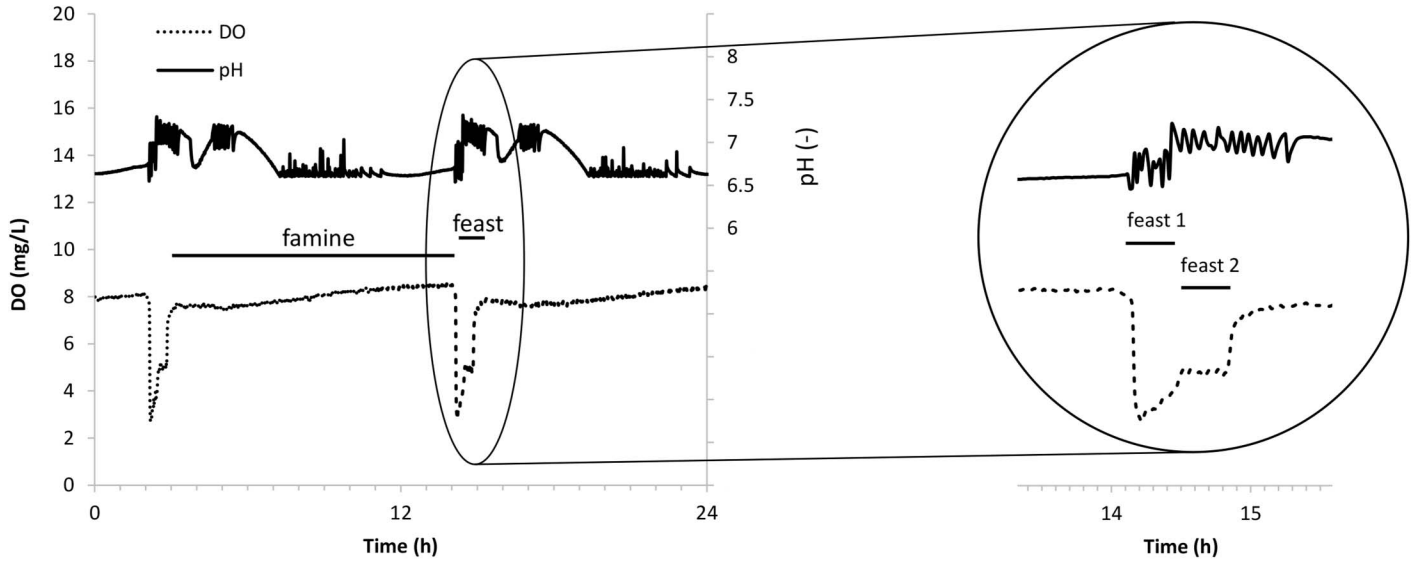

Fig. 3. Typical DO concentration and pH profiles during a period of two cycles (2 × 12 h) in the enrichment reactor.

in view of the potential polyhydroxyvalerate (PHV) content in the PHA (Albuquerque et al. 2011; Jiang et al. 2011a) and was 0.36 ± 0.02 g COD/g COD.

The NaOH consumption for maintaining the pH at 5.0 in the UASB reactor and the buffer vessel was on average 1.4 and 0.3 mol/day, respectively, while the production rate of VFA was in total 13 mol/day. Thus, the addition of base was substantially lower than the amount of VFA produced. The difference is due to the presence of a significant amount of bicarbonate alkalinity in the influent (pH 7).

Enrichment Reactor

After start up of the enrichment SBR reactor, a typical feast-famine pattern became apparent within a few cycles, with low DO concentrations during the feast phase and higher DO concentrations during the famine phase (Fig. 3). Two distinct periods could be observed during the feast phase, indicating the sequential depletion of substrates (both periods were related to VFA uptake; a more detailed analysis of the dynamics during the operational cycle is provided subsequently in this section).

Every cycle, the enrichment reactor was fed with a pulse of substrate containing on average 400 g COD of VFA per cycle, corresponding to approximately 4 mol VFA. To prevent a too-strong pH drop, the substrate was dosed intermittently while maintaining a minimum pH of 6.6. When the substrate dosage was finished (start of Feast 2 in Fig. 3), a sharp increase in pH was observed, initiating the dosing of a 1-M HCl solution in order to maintain pH below 7.2. The subsequent pH profile during the famine phase was influenced by various factors: shortly after the feast phase the pH had the tendency to increase due to stripping of CO_2 and hydrolysis of the urea in the dosed nutrients (the nutrients were dosed after the substrate dosage was finished). On average, 3.0 mol/cycle HCl was dosed in order to keep the pH below 7.2. Later in the famine phase, the pH decreased due to ammonium uptake and on average 0.36 mol/cycle NaOH was dosed to keep the pH above 6.6.

The length of the feast phase varied initially between 30 and 80 min, but became shorter and more stable (45 ± 4 min) after Day 18 of operation (Fig. 4), indicating that the process became stable in operation.

The average sludge concentration at the end of the cycle was 2.2 ± 1.2 g VSS/L and the average yield of biomass on COD was 0.38 g VSS/g COD. The SVI_{30} of the sludge at the end of the cycle was 178 ± 80 mL/g. Any observable nitrification did not occur (<1% of nitrogen conversion).

The conversions during the cycle were characterized by detailed sampling and measurement of the amounts of substrate, PHA, and VSS in the reactor. Data from a typical cycle are shown in Fig. 5.

The PHA content increased during the feast phase when VFA was present. At the end of the feast phase, all VFA was consumed and the PHA content of the biomass was 0.50 ± 0.05 g PHA/g VSS. Butyrate and valerate were depleted faster than acetate and propionate [Fig. 5(b)], corresponding to the observed dual feast phase (Fig. 3). After all VFA was depleted, there was still soluble COD present in the reactor—the amount of non-VFA COD converted was about 20% of the total amount of COD converted, the remaining 80% being VFA—but the subsequent uptake of this soluble COD in the famine phase did not increase the amount of PHA in the system. Instead, PHA was degraded while the amount

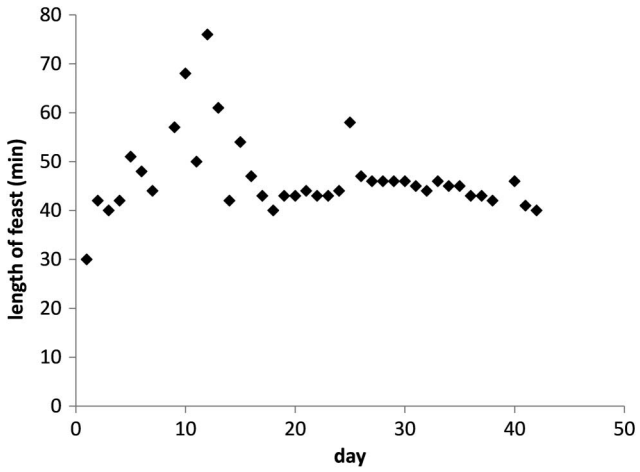

Fig. 4. Development of the length of the feast phase in the enrichment reactor evolving over consecutive cycles. The length of the feast phase was determined from the DO concentration profile, with the feast phase characterized by relatively low DO concentrations due to rapid substrate conversion (see also Fig. 3).

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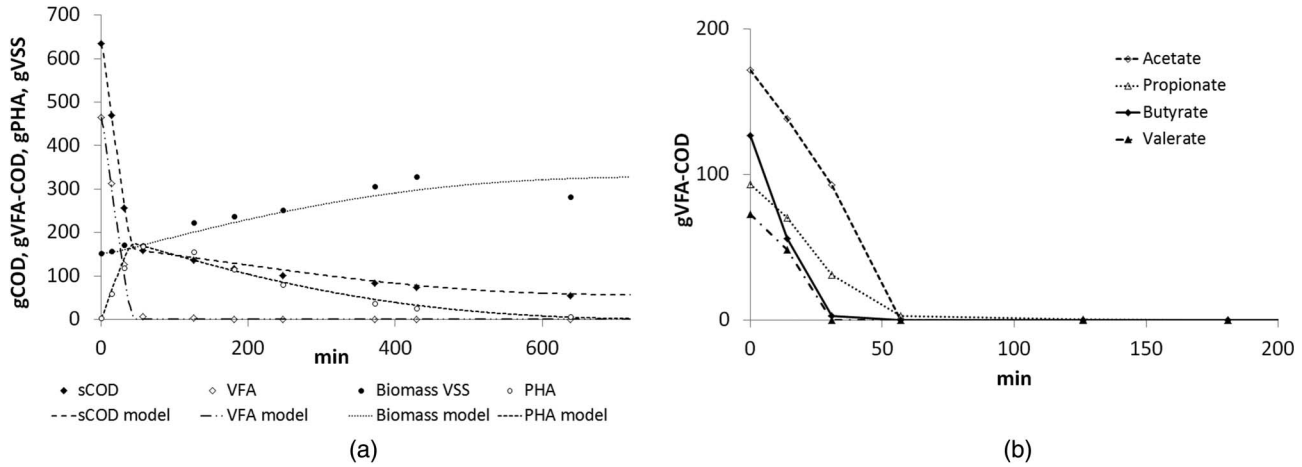

Fig. 5. Detailed characterization of a typical SBR cycle including (a) total amounts of VFA, PHA, and active biomass (X = VSS − PHA); and (b) amounts of individual VFAs. Due to changing liquid volumes during the reactor feeding, data are represented as amounts and not as concentrations.

of active biomass (i.e., VSS minus PHA) increased throughout the remainder of the cycle, indicating biomass growth on PHA but also on soluble COD degraded during the famine phase. The concentration of soluble COD in the effluent of the enrichment reactor was 0.3 ± 0.2 g COD/L.

In order to estimate characteristic process parameters, a process model (Tamis et al. 2014b) was calibrated to the experimental data from the cycle measurement. Model output indicated that most kinetic and yield parameters were comparable to values reported in earlier studies (Fig. 5 and Table 1).

Microbial Community Structure

FISH microscope images showed that *P. acidivorans* represented a significant fraction of the biomass in the reactor, although a side population comprising several morphologically distinct bacterial species was structurally present (Fig. 6). The species present in the side population were not identified in this study.

Pilot-Scale Accumulation Experiments

Accumulation experiments were conducted with biomass that was harvested from the enrichment reactor at the end of the operational cycle. Initial active biomass concentrations were around 2 g/L and initial ammonium, nitrite, and nitrate concentrations were lower than 5 mgN/L. The accumulation reactor was fed with fermented process water from the anaerobic buffer vessel during a period of 24 h. The maximum achieved PHA content after accumulation was 0.70 to 0.80 g PHA/g VSS. The molar PHV fraction of the produced PHA was around 0.25 and seemed to correspond to the molar fraction of propionate plus valerate compared with total VFA (0.26 mol/mol). The chromatogram for PHA detection had no other significant peaks, suggesting that other forms of PHA (such as polyhydroxymethylvalerate or polyhydroxyhexanoate) were not produced in relevant quantities. The conversions during a representative PHA accumulation experiment are presented in Fig. 7.

There was a remarkable decrease in the yield of PHA on VFA during the accumulation experiment: while during the first 4 h, the yield of PHA on VFA was around 0.4 g PHA/g COD, comparable to the yield that was observed in the enrichment reactor and in literature (Tamis et al. 2014a), the yield after 10 h was only around half of this value and after 24 h the yield had decreased by a factor of 10.

Discussion

Maximization of PHA Content

The microbial culture obtained in this study was dominated by *P. acidivorans* and was able to accumulate 0.7–0.8 g PHA/g VSS within 4 h, a performance comparable to results obtained earlier in a laboratory-scale study with the same process water from the same factory (Jiang et al. 2012), indicating that scale up of the PHA production process to the pilot scale did not lead to a loss in performance. The enrichment of *P. acidivorans* has been reported previously in laboratory-scale experiments with synthetic VFA substrate. Nevertheless, the maximum PHA storage capacity in the experiments on industrial process water was lower than in experiments with synthetic substrates. In order to explain the difference observed in this study, a more detailed analysis is provided in this section.

In principle, all types of wastewater containing a significant fraction of VFA may be used for the production of PHA-rich biomass (e.g., food and industry wastewaters, but also perhaps, for example, leachate from organic waste treatment facilities). Earlier studies have attributed the difference in maximum storage capacity between synthetic and real wastewater to various factors: (1) the presence of a side population with lesser PHA storage capacities, (2) the production of other types of carbon storage and/or structural compounds, and (3) the presence of solids and or fats in the influent. Essentially, all these factors can be summarized as the presence of non-VFA COD in the wastewater (Jiang et al. 2012; Tamis et al. 2014a).

Since in this study no solids or fats were present in the paper process water and there was no significant production of carbon storage other than PHA, the potential influence of side populations—the only factor left—was evaluated using a formula from an earlier study (Tamis et al. 2014b)

$$f_{\mathrm{PHA,overall}} = f_{\mathrm{PHA},1} \cdot \frac{C_{X1}}{C_{X1} + C_{X2}} + f_{\mathrm{PHA},2} \cdot \frac{C_{X2}}{C_{X1} + C_{X2}}$$

The expected overall PHA to biomass ratio ($f_{\mathrm{PHA,overall}}$) was calculated using the following assumptions: (1) a nonstoring side population (C_{X2}) proportional to the amount of non-VFA COD and corresponding with the FISH results (Fig. 6) estimated at 20%, and (2) a PHA to biomass ratio in *P. acidivorans* ($f_{\mathrm{PHA},1}$) of 9 (Johnson et al. 2009). Surprisingly, there was a distinct difference between the predicted overall PHA to biomass ratio

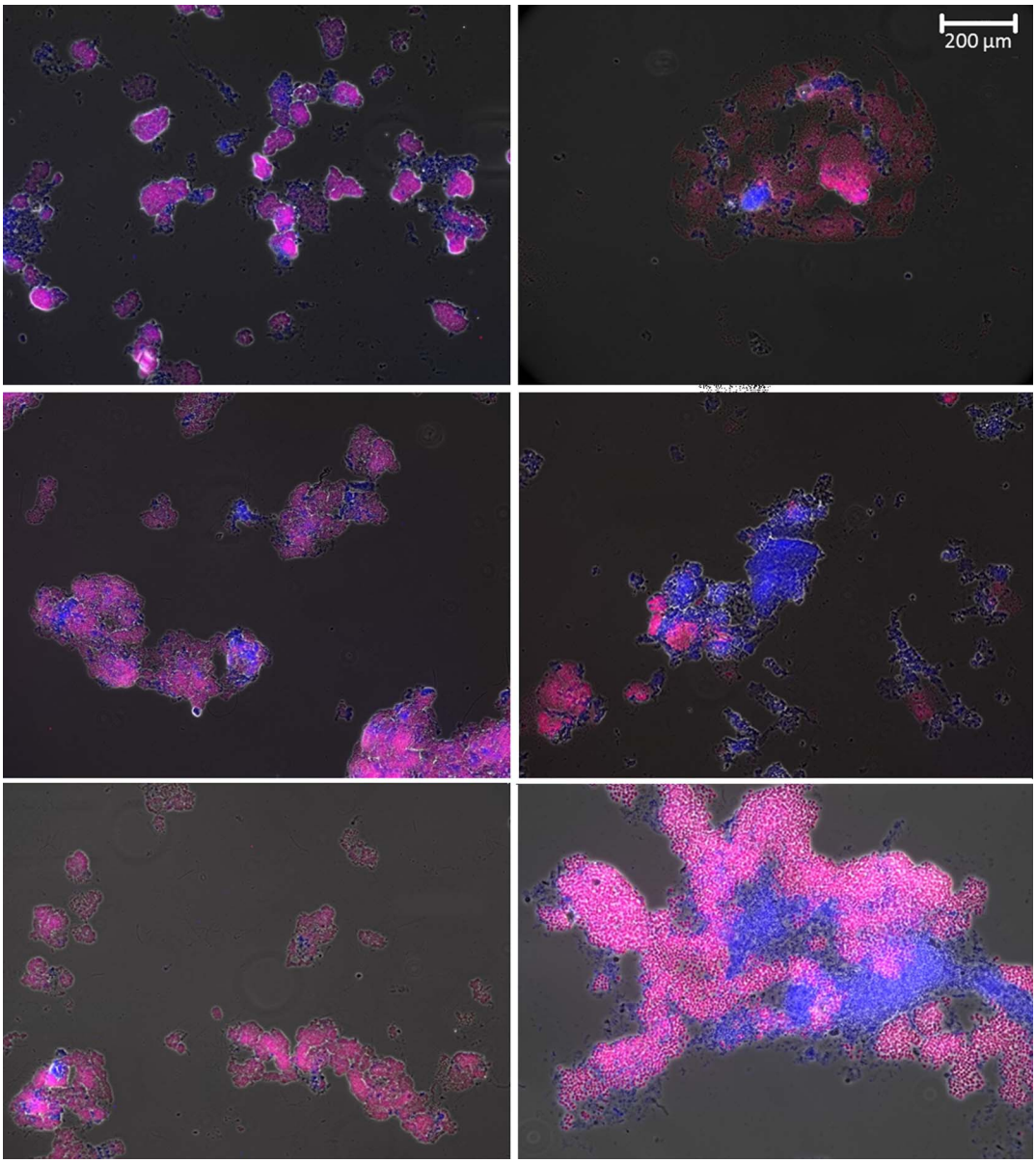

Fig. 6. Representative fluorescent is situ hybridization (FISH) microscope images of the biomass in the pilot reactor. The species specific UCB823 FISH probe was used to identify *P. acidivorans* (lighter areas), while the general EUB-338 FISH probe was used to identify other bacteria (darker areas).

(six parts PHA per part of biomass) and the observed PHA percentage (around three parts of PHA per part of biomass), suggesting that the PHA-accumulating capabilities of *P. acidivorans* are lower in a wastewater-based environment.

Optimization of the PHA Yield in the Accumulation

The yields of PHA on substrate in the enrichment reactor were similar to an earlier pilot experiment (Tamis et al. 2014a). The first 4 h the yield was around 0.4 g PHA/g COD, which is close to the theoretical maximum (Beun et al. 2002), but remarkably the yield decreases substantially after that time, declining to 0.2 g PHA/g COD after 10 h and 0.05 g PHA/g COD after 24 h (Fig. 7). Possibly, the PHA-accumulating organisms start to generate by-products or cryptic growth occurs, but this was not investigated in this study. Nevertheless, based on these observations, harvesting of the biomass in comparable full-scale operations should occur no later than 4 h in order to maintain a high PHA yield.

Outlook for Implementation

For economic and technical feasibility, the overall PHA yield on substrate is a crucial factor. The overall process yield was calculated based on a model presented by Bengtsson et al. (2008). This yield was expressed both on a COD basis and in kilograms of PHA per kilogram of substrate (Fig. 8). The parameters used were

- A yield of biomass on COD of 0.3 g VSS/g VFA-COD;
- A yield of PHA on VFA of 0.4 g PHA/g VFA-COD; and
- A final PHA content of 0.75 g PHA/g VSS.

The overall yield of the process was 0.2 kg PHA/kg COD, a significant increase compared with earlier studies (Bengtsson et al. 2008). The PHA yield can be improved further by (1) increasing the fraction of VFA after anaerobic fermentation, which can be achieved by minimizing biomass and H_2 production in the anaerobic fermentation, and (2) maximization of the PHA content in the accumulation step. In such an optimized system, an overall yield of 0.3 kg PHA/kg COD could be possible; or expressed in terms of COD yield: 0.5–0.6 kg/kg.

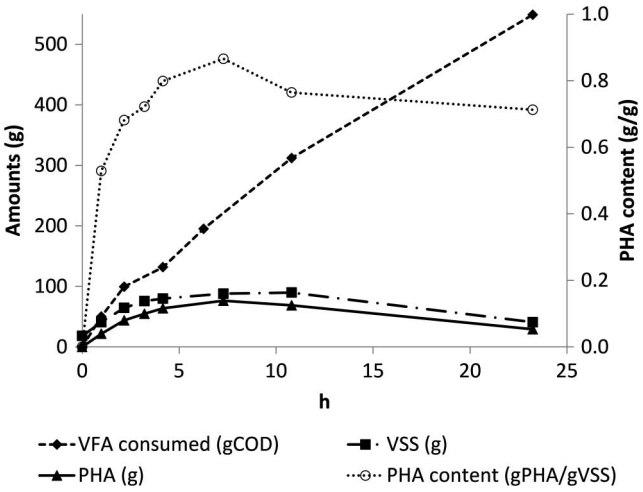

Fig. 7. Production of PHA-rich solids in the accumulation process; the lion's share of PHA was produced during the first 4 h (Day 9).

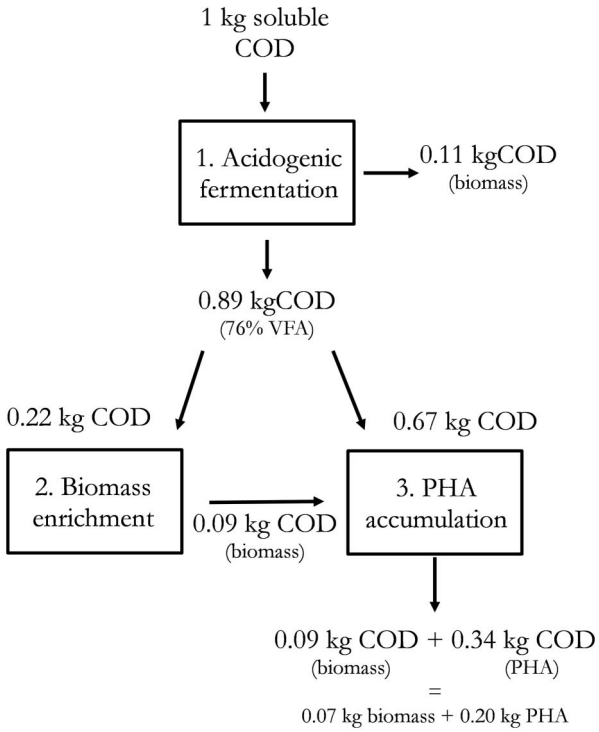

Fig. 8. Flow of COD in the PHA production process. The COD of biomass and PHA in this study were around 1.4 and 1.7 g COD/g, respectively.

The results obtained in this study also show that roughly 50% of the COD is oxidized. Assuming an energy input of 0.5 kWh/kg oxygen (Tchobanoglous et al. 2003), this would result in an energy consumption for aeration of around 1 kWh per kilogram of PHA (representing about USD 0.10/kg PHA, depending on location).

In this study, the pH in the reactors was maintained around 7. In order to get an idea of the economic impact of pH control, we calculated the costs for the unoptimized system described in this study (VFA production controlled at pH 5 and PHA reactor around pH 7) using the following assumptions:

- 20 g PHA can be produced from 100 g VFA-COD;
- 100 g VFA-COD corresponds to roughly 1 mol of VFA;
- 1 mol base is used per mole of VFA produced in the anaerobic fermentation;
- 1 mol acid is dosed per mole of VFA in the enrichment and accumulation reactors; and
- Costs for acid and base are USD 0.01 per mole.

The resulting cost for consumption of acid and base for pH control is herewith roughly USD 1 per kilogram of PHA, indicating that improving pH control strategies should be a priority for scale up. First results (Tamis, unpublished data, 2017) indicate that in many cases it should be possible to operate without pH control.

Apart from operational costs of the upstream part of the PHA production process (i.e., aeration and pH control, discussed previously), the single most important factor for successful scaling up is the development of applications for the waste-based PHA product. With this comes the potential requirement for further processing [downstream processing (DSP)]. Application development and its related downstream processing issues are suggested as the most important cost factor and an imperative future research topic. Possibly, the material could be directly used in the paper-making process, e.g., to improve water resistance. Realization of these types of direct applications of PHA will save substantial costs on downstream processing and logistics and therewith contribute to successful market introduction.

Finally, PHA-producing facilities that use wastewater in a setting such as in this study should take into account economic factors that are not only related to production of renewable biomaterials but also to the production of clean effluent water. In this study, a remaining fraction of COD was still present at the end of the process, suggesting that further investigation of posttreatment is required.

Conclusion

Pilot-scale production of PHA-containing biomass showed that a laboratory-scale strategy could be successfully translated into industrial conditions. A biomass with a PHA content of 0.70 to 0.80 g PHA/g VSS could be produced within 4 h. Factors influencing implementation were assessed in this study: (1) the laboratory-scale process was optimized for implementation by showing that it is possible to work without expensive additives [allylthiourea (ATU)] to prevent nitrification, (2) an overall process yield of 0.2 kg PHA per kilogram COD treated was estimated, and (3) results from this study indicate that pH control implies a major cost factor and that operational strategies without—or perhaps with less strict—pH control will significantly reduce PHA production costs.

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Supplemental Data

A description of the process model is available online in the ASCE Library (www.ascelibrary.org).

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