Toward Polyhydroxyalkanoate Production Concurrent with Municipal Wastewater Treatment in a Sequencing Batch Reactor System

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Abstract: Bacteria can synthesize cytoplasmic granules known as polyhydroxyalkanoates (PHAs), which are carbon and energy storage reserves, from organic carbon when subject to stressful environmental conditions. PHAs are also biodegradable thermoplastics with many potential commercial applications. The purpose of the research reported herein was to evaluate the feasibility of integrating PHA production within a municipal wastewater treatment (WWT) configured as a sequencing batch reactor (SBR). Four bench-scale WWT SBRs were tested at decreasing organic loading rates to assess the potential to enrich for microbes capable of feast/famine PHA synthesis. For each treatment SBR, sidestream batch reactors receiving higher quantities of primary solids fermenter liquor were operated to produce PHA. Results from this study demonstrate that a treatment SBR supplied moderate strength wastewater can enrich for the target microorganisms, with PHA yields of 0.23–0.31-mg PHA per mg chemical oxygen demand, and produce high quality effluent. In sidestream batch reactors, microorganisms that fed excess quantities of substrate can rapidly synthesize significant quantities of PHA. Based on the results of this study, we estimate that a 1 million gallon per day SBR WWT-PHA production system could generate 11–36 t (12–40 t) of PHA annually.

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Introduction

Polyhydroxyalkanoates (PHAs) are biologically produced, biodegradable thermoplastics with many potential commercial applications (Chen 2009). Bacteria synthesize PHAs, which are carbon and energy storage reserves, as cytoplasmic granules from organic carbon when subject to stressful environmental conditions (Lee 1996; Serafim et al. 2008). PHA biosynthesis, which is accomplished by over 300 bacterial species (Lee 1996), is stimulated by either excess soluble carbon with a concurrent macronutrient limitation (typically limited on either nitrogen or phosphorus), a limitation in a terminal electron acceptor [e.g., oxygen, commonly associated with enhanced biological phosphorus removal (Fuhs and Chen 1975)], or a so-called feast/famine environment wherein microorganisms realize a transient excess of soluble carbon without any nutrient limitations (Dionisi et al. 2004a). Poly-3-hydroxybutyrate (PHB or P3HB) was the first PHA discovered (over 75 years ago), and hence is the most extensively characterized type (Lemoigne 1926; Madison and Huisman 1999). Since this initial discovery, many forms of hydroxyalkanoic monomer units have been identified (Madison and Huisman 1999).

Common precursors to PHA synthesis include organic acids such as acetic and propionic acid. The type of carbon substrate dictates the polymeric structure of the PHA (Madison and Huisman 1999), with some of the most commonly studied forms including PHB, polyhydroxyvalerate (PHV), and poly-4-hydroxybutyrate. In turn, each form of PHA yields different polymer properties. PHB exhibits similar properties to polypropylene including melting temperature and crystallinity, but the polymer is brittle upon crystallization and exhibits little stress resistance (Madison and Huisman 1999). Polymer improvements have been accomplished through copolymerization with PHV to increase ductility and impact resistance and lower processing temperatures (Madison and Huisman 1999). PHB-co-HV copolymers have numerous applications including potential use in biodegradable films and utensils (Anonymous 1989; Madison and Huisman 1999) and in medical applications (Zinn et al. 2001). polyhydroxybutyrate-co-valerate (PHBV) at the 10% HV content has properties comparable to high-density polyethylene (e.g., milk jugs) while at a 20% HV content the properties are approaching that of low-density polyethylene (e.g., flexible film). Furthermore, unrefined PHA can potentially be used to produce wood-plastic composites (Coats et al. 2008).

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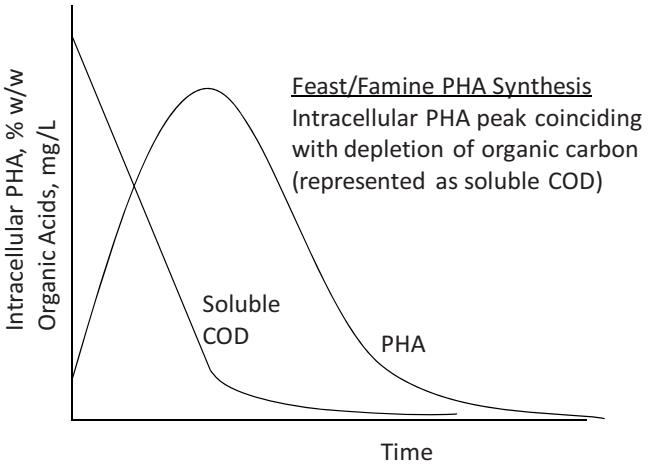

Fig. 1. Conceptual feast/famine PHA synthesis curve. As shown, peak intracellular PHA concentration occurs concurrent with depletion of readily biodegradable organic carbon (represented as soluble COD).

PHA is currently produced commercially using pure or recombinant microbial cultures grown on refined substrate from food crops (e.g., glucose from corn) under sterile conditions (Braunegg et al. 1998). However, similar to the concerns in using valuable cropland for producing biofuel feedstock (Roberts et al. 2010), the use of food crops for PHA production poses long-term problems; the overall environmental footprint of the current process has also been questioned (Gerngross 1999; Gerngross and Slater 2000). PHA could alternately be produced using mixed microbial consortia under nonsterile conditions. Numerous studies have examined PHA cultivation using microorganisms from activated sludge wastewater treatment (WWT) facilities that fed synthetic wastewater with promising results (Beun et al. 2002, 2000; Dionisi et al. 2005b, 2004a,b; Harper et al. 2005; Perez-Feito and Noguera 2006; Serafim et al. 2004). As a further process refinement, some investigators have examined the potential to use organic waste streams as feedstock (Beccari et al. 2009; Dionisi et al. 2005a; Rhu et al. 2003). In this proposed configuration, PHA production would be achieved by inducing feast/famine synthesis in a two-stage sequencing batch reactor (SBR) by feeding microorganisms' organic acid-rich wastewater. The first stage reactor would enrich for a microbial consortium capable of storing PHA, while commercial quantities of PHA would be produced in a second stage reactor.

An extension of PHA production on waste organics would integrate the process within municipal WWT to concurrently produce both PHA and treated wastewater. With this approach, polymer production could enhance the economics of WWT. Moreover, the concept has potential universal application since WWT is broadly mandated and municipal waste streams will continually be generated. The concept would center on operating an activated sludge wastewater treatment plant (WWTP) in a SBR mode to enrich for microorganisms capable of feast/famine PHA synthesis (a typical feast/famine PHA synthesis response is depicted in Fig. 1). The feast/famine response is broadly recognized as an efficient means to produce PHA using mixed microbial consortia within aerobic systems (Serafim et al. 2008). PHA production could occur in either a single-stage SBR or in a two-stage system as described by Dionisi et al. (2005a). In this regard, our research group has previously conducted proof-of-concept investigations for a combined PHA-WWT process (Coats et al. 2007). Treatment SBRs that fed 100% municipal primary solids fermenter liquor in a feast/famine environment maintained a mixed microbial consortium capable of storing intracellular PHA at 10–25% dry weight (d.w.) under either anaerobic/aerobic cycling or strictly aerobic conditions. Peak intracellular PHA storage in a second stage batch reactor reached 50–55% d.w.

While our preliminary results were encouraging, our initial research did not consider how to best operate the system (single stage versus two stage) to maximize use of and recovery of the influent carbon for concurrent PHA production and WWT. We envision two scenarios that could be employed to achieve PHA production within a WWTP. Scenario 1 would integrate treatment and PHA production within a single SBR [Fig. 2(a)] while Scenario 2 would separate the two processes [Fig. 2(b)]. SBRs are a reactor configuration commonly employed in full-scale WWTPs (Kirschenman and Hameed 2000; Poltak 2005; Tchobanoglous et al. 2003). The SBR process configuration would allow the method of substrate addition (all at the beginning of a cycle) to be manipulated on the full scale to induce feast/famine PHA synthesis (i.e., Fig. 1). To increase the quantity of soluble PHA precursors in the influent wastewater, both scenarios would integrate a continuous flow in-line primary solids fermenter. A significant fraction of organic carbon entering a municipal WWTP is associated with primary solids (Tchobanoglous et al. 2003), and this organic matter can be readily fermented to PHA precursors (Bouzas et al. 2007). In the treatment reactors the influent organic carbon would first drive cell growth processes while concurrently enriching for microorganisms capable of feast/famine PHA synthesis. Excess carbon above that required for treatment would fuel PHA synthesis, either within the treatment reactor (Scenario 1) or in a sidestream reactor (Scenario 2). For Scenario 2, the sidestream reactor would be operated only long enough to maximize PHA production; with the microbes conditioned for feast/famine PHA synthesis, it is anticipated that polymer production would commence immediately following the addition of substrate.

The purpose of the research described herein was to determine which scenario, or if both scenarios, could be considered for implementation. We operated laboratory-scale batch reactors to simulate both scenarios. The objectives of this research were to (1) establish the minimum organic carbon loading rate required to enrich for a mixed microbial consortium capable of concurrent feast/famine PHA synthesis and WWT; (2) evaluate the effect of organic loading rate (OLR) on the quantity of PHA produced; (3) identify an optimal operational scheme to achieve commercial production; and (4) estimate the potential PHA production capacity of a full-scale WWT process. All research activities were conducted using mixed microbial consortia and real wastewater.

Materials and Methods

Microorganism and Substrate Sources

A microbial consortium was collected from the anoxic basin at the Lincoln, Calif., WWTP, which is a biological nutrient removal facility. The consortium was used to inoculate both the bench-scale reactors and the primary solids fermenter. Raw wastewater was obtained from the same facility on a weekly basis. Thickened primary solids for the bench-scale fermenter were collected on a weekly basis from the primary clarifiers at the city of Davis, Calif., WWTP, a facility that uses bar screens and aerated grit

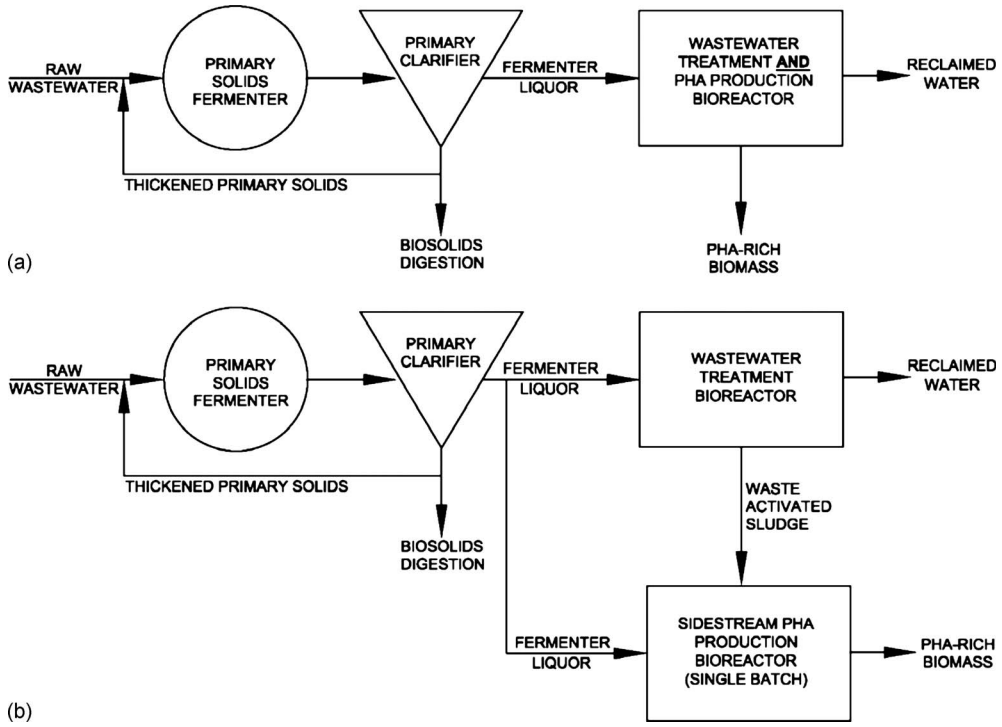

Fig. 2. Schematic diagram for conceptual WWT-PHA production processes. As shown, both scenarios would integrate an in-line primary solids fermenter: (a) Scenario 1: WWT and PHA production would occur within a common SBR; (b) Scenario 2: WWT would occur within a SBR, while PHA production would occur in a sidestream batch reactor.

removal upstream of primary sedimentation. Both the raw wastewater and thickened primary solids were stored at 4 °C prior to use.

Bench-Scale Primary Solids Fermenter Operation

A 15-L bench-scale municipal primary solids fermenter was operated continuously at room temperature (approximately 22–23 °C) for over 12 months to produce a waste stream rich in volatile fatty acids that could be used for PHA synthesis. The fermenter was constructed of cylindrical high-density polyethylene and was continuously stirred using a low speed mixer (Arrow Engineering, Hillside, N.J.) at 60 rpm. The 24-h batch feed cycle consisted of a single decant (4 L) and feed [1,600-mL thickened primary solids and 2,400-mL deionized (DI) water] at the start of each cycle. Influent thickened primary solids ranged from 2 to 4% concentration (w/v) as per data obtained from WWTP staff. The fermenter hydraulic retention time and solids retention time (SRT) were maintained at 3.75 days (no settling phase). Fermenter decant was centrifuged at 10,000 g, with the resulting liquor (fermentate) used either immediately or stored at 4 °C to serve as feedstock for the PHA reactors.

Bench-Scale WWT Reactor Operation

To simulate the proposed full-scale PHA-WWT scenarios (i.e., those depicted in Fig. 2) in the laboratory, four bench-scale WWT SBRs (Reactors A–D) were operated for 90 days, each at different mean organic load rates (OLRs) (Table 1). The SBRs were operated on a 24-h cycle, with WWT focused on organic carbon and ammonia-nitrogen removal. Considering the challenge in operating a bench-scale continuous flow fermenter and further recognizing that effluent from the bench-scale batch fermenter would generate a higher strength effluent stream than could be realized in a continuous flow fermenter, raw wastewater was mixed with fermenter liquor to generate variable OLRs to the treatment reactors (Table 1). The OLRs were selected because they bracketed real potential for a full-scale in-line fermenter (Bouzas et al. 2007). OLRs were calculated as the average mass of substrate chemical oxygen demand (COD) added to the reactor divided by the total reactor volume and were based on the mean soluble COD concentration in the (1) primary solids fermentate and (2) raw wastewater over the 90-day operational period.

The reactors were constructed of 8-in.-diameter acrylic tubing with a Sanitaire 7-in. fine bubble diffuser (Brown Deer, Wis.) mounted to the bottom of the tubing section to provide mixing and aeration. The reactors had a 4-L working volume, with decant and feed (750 mL) occurring once daily at the start of a cycle. Prior to decanting effluent, tap water was added to account for evaporation. Biomass was wasted with the daily decant, and the SRT was maintained at 5.33 days. SBR sidewalls and diffuser surfaces were scrubbed before the daily decant to prevent excess solids accumulation on interior surfaces. Prior to any sampling, reactors were operated for a period of six SRTs to ensure that steady-state conditions prevailed.

Sidestream PHA Reactor Operations

Sidestream aerobic PHA production batch tests were performed using biomass obtained from each of the treatment SBRs. The purpose of these tests was to establish sidestream PHA production potential. Mixed liquor was wasted from each treatment SBR at the end of a cycle and combined with specific volumes of fermenter liquor. For these investigations, the OLR was modulated on a food-to-microorganism (F/M) basis. As with the treatment

Table 1. Summary of Typical WWT SBR Operating Parameters

	Reactor A	Reactor B	Reactor C	Reactor D
Solid retention time (SRT) (day)		5.33		
Substrate (% fermenter liquor+% raw wastewater, v/v)	100/0	50/50	25/75	10/90
Average OLR,[a] mg COD (L day)$^{-1}$	575	342	167	92
Reactor temperature (°C)	19.5–22.7	20.2–22.8	19.8–22.7	19.9–22.7
pH range over a cycle	7.8–9.3	7.9–9.0	7.8–8.8	7.8–8.9
MLVSS[b] range (g L^{-1})	1.6–2.6	1.0–1.6	0.6–0.8	0.4–0.6
Average F/M ratio[c] (day^{-1})	0.33	0.29	0.33	0.26
COD:N ratio (at $t=0$ within a cycle)	52–55	37–48	26–36	16–27
SVI	51.1	61	174	206

[a]The average OLRs were calculated using the mean COD concentration in the substrate (i.e., primary solids fermentate plus raw wastewater) over the 90-day operational period.

[b]MLVSS. The range of MLVSS is largely attributed to variations in the OLRs arising from variation in the thickened solids concentration obtained from the primary clarifiers that in turn influenced the COD concentration of the primary solids fermentate.

[c]F/M calculated by dividing mass of COD by mass of MLVSS.

reactors, the F/M ratio was calculated as the mass of COD added divided by the mass of mixed liquor volatile suspended solids (MLVSSs) obtained from the treatment reactors. The fermenter liquor COD and the treatment reactor MLVSS were determined prior to operating sidestream reactors, and the volume of fermenter liquor added to the wasted MLVSS was adjusted to yield the target F/M ratios (Table 2). Biomass and substrate were combined within 15 min of withdrawal from the respective treatment reactors. Tests were conducted in 1-L glass beakers at room temperature (approximately 23°C) using bubbling stones and stir plates to ensure aerobic and well-mixed conditions. The dissolved oxygen (DO) was maintained >2 mg L^{-1}. For each F/M ratio, the maximum intracellular PHA content within the biomass (% d.w.) and total yield (mg PHA per mg soluble COD consumed) were determined.

Analytical Methods

Soluble carbon utilization was measured by the COD test in accordance with Standard Methods 5220-D (Clesceri et al. 1998), using Hach high-range ampules (Hach Company, Loveland, Colo.). Total phosphorus was determined using Hach Method 8190, which is equivalent to Standard Methods 4500-PE (Clesceri et al. 1998). Ammonia-nitrogen was measured using a continuous flow Timberline Model 383 inorganic nitrogen analyzer (Timberline Instruments, Boulder, Colo.). Samples for soluble constituents were filtered through 0.22-μm filters (Millipore Corp., Billerica, Mass.) prior to analysis. Mixed liquor suspended solids and MLVSS were determined gravimetrically in accordance with Standard Methods 2540 (Clesceri et al. 1998). Sludge volume index (SVI) was also determined according to Standard Methods.

Prior to intracellular PHA analyses, biomass was chlorinated with 6.15% sodium hypochlorite solution (Clorox Company, Oakland, Calif.) for 5 min and centrifuged at 10,000 g for 10 min to obtain a pellet. The pellet was released using DI water and dried at 60°C for 4 h. The PHA content of the biomass was determined through gas chromatography/mass spectroscopy (GC-MS) (Model 6890-5973N, Agilent, Palo Alto, Calif.) following the method of Braunegg et al. (Braunegg et al. 1978). Briefly, dried samples were digested at 100°C in 2 mL each of acidified methanol (3% v/v sulfuric acid) and chloroform. Benzoic acid was added to the chloroform as an internal standard. Following vigorous vortexing of the mixture with 1-mL DI water, the organic layer was recovered for analysis. PHA standards [poly(3-hydroxybutyric acid) and poly(3-hydroxybutyric acid-*co*-3-hydroxyvaleric acid) (Fluka and Aldrich, St. Louis, Mo.)] were similarly processed for analysis. GC-MS was performed with split injection under an initial oven temperature of 40°C (2 min) ramped up to 200°C at 5°C min^{-1} using a 30-m ZB-624 column (0.25-mm inside diameter, 1.4-μm film; Phenomenex, Torrance, Calif.). PHA was confirmed by retention time and mass spectral matching with the

Table 2. Summary of WWT and PHA Production Performance in the WWT Reactors

	Reactor A	Reactor B	Reactor C	Reactor D
Peak % PHA during cycle[a] (% d.w.)	10.4	10.1	9.6	8.4
Increase in PHA content (% d.w.)	7.2	6.6	4.6	3.5
Average PHA produced (mg)	568	340	135	64
Average PHA yield, mg PHA (mg COD)$^{-1}$	0.31	0.31	0.23	0.25
Average effluent soluble COD (mg L^{-1})	115	41	63	40
Average COD removal (%)	83	89	73	69
Average effluent phosphorus (mg L^{-1})	1.7	1.95	4.6	4.0
Average phosphorus removal (%)	76	62	4	3
Average effluent ammonia-nitrogen (mg L^{-1})	0.65	0.15	0.20	0.15
Average ammonia-nitrogen removal (%)	95	98	97	98
Sludge production,[b] g MLVSS (g COD)$^{-1}$	0.76	0.93	0.91	0.84

[a]PHA (mg) divided by TSS (mg) on a percent d.w. basis. Results shown are the average of the two sampling events.

[b]Sludge production calculated by dividing the average daily MLVSS concentration by the average daily COD removal.

PHA standards as methyl ester derivatives and quantified based on the internal standard. Total cellular PHA content was calculated on a percent dry cell weight basis [i.e., (mass of PHA/mass of biomass) $\times$ 100, % d.w.].

PHA yield was determined as mg PHA produced per mg substrate (as COD) consumed. The adjusted PHA yield was determined as mg PHA as COD per mg COD consumed. The respective masses of PHB and PHV were converted to a COD basis according to Dionisi et al. (2005a). The F/M ratio was calculated as the mass of COD added to a reactor divided by the mass of MLVSS prior to substrate addition.

Results and Discussion

Primary Solids Fermenter Operation

The fermenter consistently produced substrate with soluble COD concentrations between 1,700 and 3,000 mg L^{-1}. The variability in soluble COD was attributable to the fluctuation in influent primary solids concentration (2–4% w/v). Centrifuged fermenter liquor pH was 4.5–5. Specific forms of organic acids in the fermentate were not determined. However, based on research by others in our research group (unpublished data), the predominant forms of organic carbon in the fermenter liquor would be acetic and propionic acid. Bouzas et al. (2007) made similar observations in a recent primary solids fermenter optimization study.

Concurrent PHA Synthesis and WWT

The consortia in the four SBRs (Table 1) consistently stored carbon as PHA at approximately 8–11% d.w. PHA (Table 2). Peak PHA accumulation occurred concurrent with the depletion of readily biodegradable COD (rbCOD) (i.e., feast/famine PHA synthesis) and approximately 1–2 h into the aerobic period. These results were consistent with our prior investigations (Coats et al. 2007). rbCOD was assumed to be the difference between initial bioreactor soluble COD and residual soluble COD. A comprehensive review of the PHA chromatograms showed that only PHB and PHV were synthesized, with PHB-co-HV copolymer ratios of approximately 50:50–70:30 (%, w/w).

Average PHA yield (mg PHA synthesized per mg soluble COD used) generally improved with increased COD:N ratio (Table 2). This effect of nitrogen deficiency on PHA synthesis is well known, and the polymer yields presented herein are comparable to that observed by Punrattanasin et al. (2006) under nitrogen limiting conditions. Also, polymer yields increased with increasing OLRs. While OLR increased 82% from Reactor D to C, the quantity of PHA produced increased 110%. OLR increased 105% from Reactor C to B, and PHA production increased approximately 152%. Finally, the OLR increased 68% from Reactor B to A, while polymer production increased by approximately 67%. Thus, it appears that the microorganisms shunted proportionally more of the carbon to storage (rather than growth) as OLR increased.

Regarding WWT, all four reactors removed >69% of soluble COD (Table 2), with maximum removal of 89% in the reactor that fed 50% fermenter liquor (Reactor B). Moreover, in all treatment reactors the soluble COD concentrations reached steady-state concentration by the end of the operational cycle, indicating that maximum soluble organic carbon removal occurred. Biomass from Reactors A and B showed excellent settling properties each with SVI below 100, while Reactors C and D exhibited less efficient settling characteristics, with SVIs over 150. Note that the influent COD:N ratio (Table 1) was substantially lower in Reactors C and D, as contrasted with the other two treatment reactors. The reduced SVIs in Reactors C and D may thus have been a result of reduced quality substrate (i.e., receiving less fermenter liquor), which could have contributed to the growth of filamentous microbes. Low carbon substrate is commonly associated with this type of problem at full-scale WWTPs (Jenkins et al. 2003). Regarding inorganic nutrient removal, Reactors A and B achieved >57% P removal over a cycle and produced effluent at <2 mg P L^{-1} (Table 2). However, Reactors C and D achieved little phosphorus removal. Significant ammonia-nitrogen removal occurred over a full cycle in all reactors, with negligible effluent concentrations (Table 2). These results suggest that, in addition to microorganisms capable of feast/famine PHA synthesis, a relatively robust population of autotrophic microorganisms also developed in all four treatment reactors.

Sidestream PHA Production: Effect of F/M on PHA Storage

In the treatment reactors, peak intracellular PHA concentrations were observed within an operational cycle and not at the end of a cycle, which presents inherent challenges in recovering PHA while maintaining treatment process stability. Furthermore, in regard to polymer recovery and processing, higher intracellular PHA concentrations would also be desirable in a commercial setting. To improve PHA production and overall process efficiency, a portion of the primary solids fermentate could be directed into the WWT SBR to stimulate the growth of microorganisms capable of feast/famine PHA synthesis, and then the cells wasted from the WWT SBR could be diverted into a sidestream SBR that fed the remaining portion of the primary solids fermentate [Scenario 2; Fig. 2(b)]. In this operational scenario, PHA-rich biomass in the sidestream reactor would be recovered at peak cellular polymer concentration. By subjecting treatment reactor biomass to an increased quantity of organic carbon-rich substrate, the microbial consortium would direct most, if not all, of the organic carbon to PHA.

On the laboratory bench scale, biomass from each treatment SBR was evaluated for potential PHA production in sidestream configurations operated under aerobic conditions. As with the treatment reactors, the OLR was the manipulated variable. However, in this case the OLR was calculated as the F/M ratio, with imposed ratios ranging from 0.5:1 to 2:1 (Table 3). In all sidestream reactors tested, feast/famine PHA synthesis was observed, with peak PHA accumulation again corresponding to depletion of rbCOD (defined as the soluble COD consumed). For example, with seed biomass from Reactor A at a F/M ratio of 0.5, bulk solution rbCOD was depleted and peak intracellular PHA was observed after 1 h [Fig. 3(a)], while at a F/M ratio of 1 the two coincided at approximately 2 h [Fig. 3(b)]. However, as the F/M ratio increased the microorganisms required more time to deplete rbCOD and synthesize maximum PHA. At a F/M ratio of 2, rbCOD was depleted and intracellular PHA peaked between 5 and 7 h with biomass from Reactor A [Fig. 3(c)], between 3 and 5 h with biomass from Reactor B [Fig. 3(d)], and at approximately 7 h for Reactors C and D [Fig. 3(e)]. Finally, as the F/M ratio increased, peak intracellular PHA concentration and production also increased in all sidestream reactors (Table 3). This effect of F/M ratio was consistent with that observed by others using both

Table 3. Summary of Sidestream Aerobic Batch PHA Production Reactor Performance; Biomass from Each Treatment Reactor (A–D) Was Evaluated for PHA Production in the Sidestream Configuration

		F/M ratio[a]		
Treatment reactor	Measures of performance	0.5	1.0	2.0
A	Peak PHA content (% d.w.)	12.5	17.0	20.2
	Increase in PHA content[b] (% d.w.)	5.2	11.7	15.2
	Maximum PHA concentration (mg L^{-1})	351	356	446
	PHA produced (mg)	146	245	336
	Yield,[c] mg PHA (mg COD)$^{-1}$	0.25	0.27	0.22
	Adjusted yield,[d] mg COD (mg COD)$^{-1}$	0.38	0.41	0.33
	Initial pH	8.2	8.3	5.7
B	Peak PHA content (% d.w.)	12.2	14.3	27.5
	Increase in PHA content[b] (% d.w.)	4.7	8.3	23.0
	Maximum PHA concentration (mg L^{-1})	198	228	489
	PHA produced (mg)	76	132	409
	Yield,[c] mg PHA (mg COD)$^{-1}$	0.20	0.18	0.34
	Adjusted yield,[d] mg COD (mg COD)$^{-1}$	0.30	0.27	0.51
	Initial pH	8.2	7.8	6.2
C	Peak PHA content (% d.w.)	—	19.9	28.7
	Increase in PHA content[b] (% d.w.)	—	14.2	21.6
	Maximum PHA concentration (mg L^{-1})	—	199	347
	PHA produced (mg)	—	142	261
	Yield,[c] mg PHA (mg COD)$^{-1}$	—	0.28	0.33
	Adjusted yield,[d] mg COD (mg COD)$^{-1}$	—	0.42	0.49
	Initial pH	—	7.9	6.1
D	Peak PHA content (% d.w.)	—	19.0	23.0
	Increase in PHA content[b] (% d.w.)	—	13.2	15.2
	Maximum PHA concentration (mg L^{-1})	—	145	205
	PHA produced (mg)	—	101	135
	Yield,[c] mg PHA (mg COD)$^{-1}$	—	0.29	0.24
	Adjusted yield,[d] mg COD (mg COD)$^{-1}$	—	0.43	0.36
	Initial pH	—	7.3	5.4

[a]Calculated by dividing mass of COD added by mass of MLVSS prior to substrate addition.

[b]Calculated as maximum cellular content less the cellular content at $t=0$.

[c]Yield=increase in PHA content/(COD consumed).

[d]Adjusted yield is calculated by converting the PHB and PHV present in the biomass to COD equivalents, 1.38 and 1.63, respectively (Dionisi et al. 2005a).

a range of refined and unrefined feedstocks in aerobic single-run batch reactors (Chua et al. 2003; Coats et al. 2007; Dionisi et al. 2005a).

Although the increased sidestream reactor F/M ratios resulted in higher intracellular PHA concentrations (that is, for each respective treatment reactor biomass), results on overall PHA yield and adjusted PHA yield were mixed (Table 3). For Reactors A and D (treatment reactors with the highest and lowest OLRs, respectively), polymer yield in the sidestream reactors generally decreased at higher F/M ratios, while the opposite was observed using biomass obtained from Reactors B and C. A closer examination of the PHA synthesis trends [Figs. 3(a–e)] suggested that the respective consortia potentially metabolized carbon differently as the F/M ratio increased in each sidestream reactor. For example, for the Reactor A consortium, at F/M ratios of 0.5 and 1 the storage rate was seemingly constant up to the time point for peak PHA accumulation [i.e., relatively constant rate of PHA synthesis; Figs. 3(a and b)]. However, a different storage pattern was exhibited at a F/M ratio of 2 [Fig. 3(c)]; for the 5/25 sampling event the rate of PHA synthesis was generally low from 0 to 2 h, high from 2 to 4 h, then low again from 4 to 5 h. For the 4/30 sampling event the PHA storage rate was similarly low for the first 4 h and then increased substantially from 4 to 7 h. The average yield for this sidestream scenario was approximately 20% less than at a F/M of 1 (0.22 versus 0.27). Microorganisms from Reactors B–D exhibited similar dynamics at F/M ratios of 2 [Figs. 3(d and e)]. The cause of this response is not entirely clear. However, these sidestream reactor results suggest that saturation of feast-famine PHA metabolisms can occur when microbes are fed too much substrate all at once, and that the microorganisms must synthesize additional proteins under elevated F/M ratios before more PHA can be produced. To overcome this apparent metabolic limitation, Serafim et al. (2004) pulse fed substrate in accordance with DO concentrations, ensuring carbon availability over a longer time period without impairing the feast/famine response. The advantage of using DO for process monitoring centers on the implicit biochemical oxidation-reduction reaction between organic carbon and oxygen. Beun et al. (2002) observed a similar phenomenon for PHA production by mixed microbial consortia.

In addition to potential metabolic-level inhibition, pH may have also affected PHA synthesis at elevated F/M ratios in the

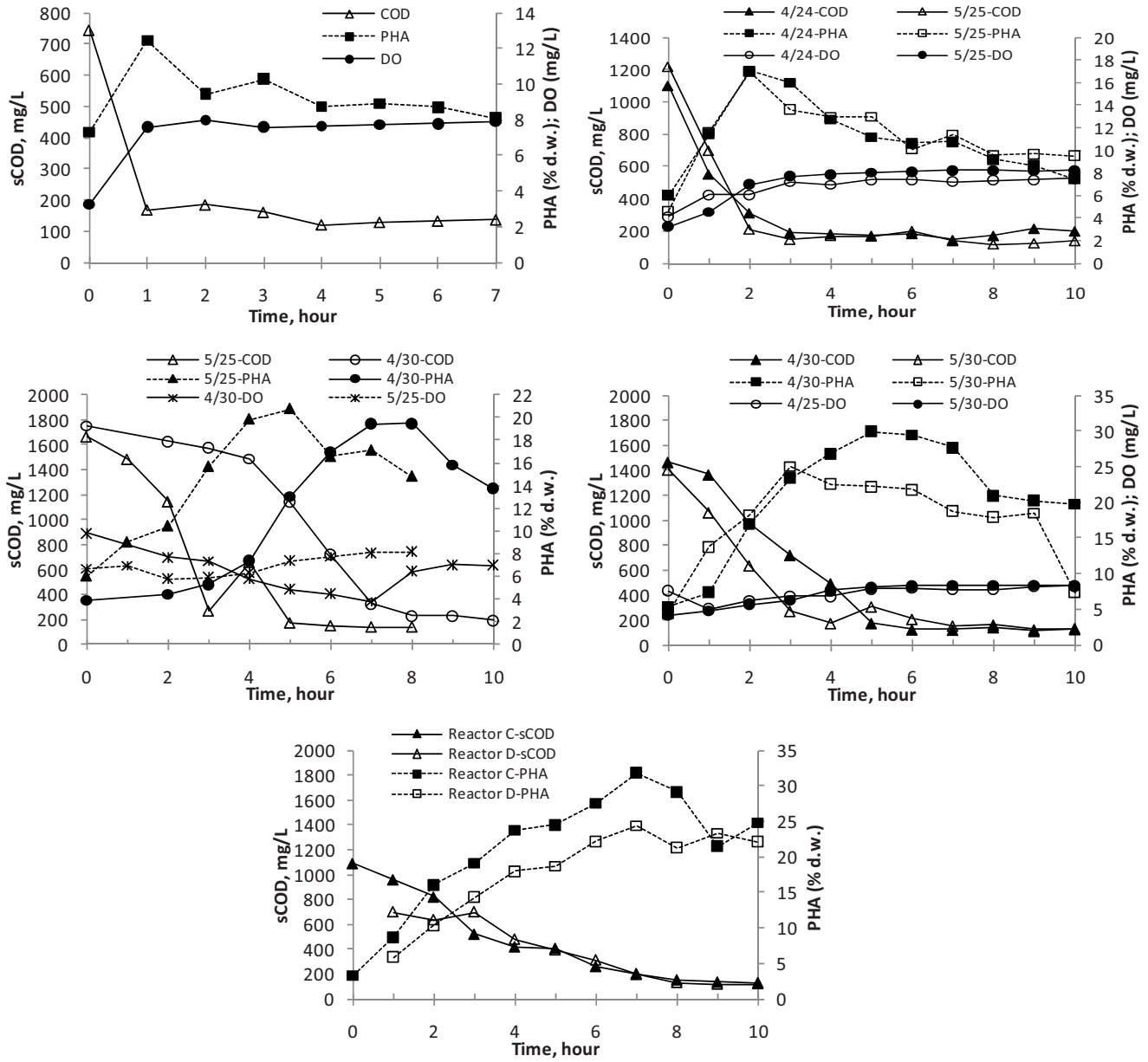

Fig. 3. Typical COD, PHA, and DO profiles in select sidestream reactors at variable F/M ratios. Results are representative of observations in all sidestream reactors. (a) F/M=0.5, results for a sidestream reactor seeded with biomass from Reactor A; (b) F/M=1, results for a sidestream reactor seeded with biomass from Reactor A; (c) F/M=2, results for a sidestream reactor seeded with biomass from Reactor A; (d) F/M=2, results for a sidestream reactor seeded with biomass from Reactor B; and (e) F/M=2, results for a sidestream reactor seeded with biomass from Reactors C and D.

sidestream reactors. As shown in Table 3 and Fig. 4, the initial sidestream reactor pH at $t=0$ decreased from >7.3 at F/M ratios of 0.5 and 1 to <6.2 at a F/M ratio of 2:1. Commensurately, the initial rate of substrate utilization and PHA synthesis at elevation F/M ratios decreased [Figs. 3(a–e), comparatively]. For example, during the first hour of operation in sidestream Reactor A the specific rate of substrate utilization decreased from approximately 200-mg COD (g MLVSS h)$^{-1}$ at a F/M of 0.5 to approximately 44 at a F/M of 2; the initial pH decreased from 8.2 to 5.8. Similarly, the consortium in sidestream Reactor B realized a reduction in the specific rate of substrate utilization from approximately 220 to 120, while the initial pH dropped from 8.2 to 6.2. Moreover, sidestream batch tests at F/M ratios of 3:1 and 4:1 showed no apparent PHA storage response, as reflected by minimal COD consumption during the 10-h test period (data not shown); in these sidestream reactor tests the initial pH was 4.6–4.7. Dionisi et al. (2005a) observed that maximum PHA accumulation with mixed consortia occurred between a pH of 6.5 and 8.5, which is consistent with our observations. Chua et al. (2003) also observed that when raw municipal wastewater was used to acclimate sludge for aerobic batch PHA production, minimal PHA production was observed at pH 6 and 7. Pulse feeding substrate to enhance PHA synthesis and yield, as discussed above, would likely serve to mitigate pH effects.

Finally, it was observed that PHA yield in all sidestream Reactor A configurations never approached that observed in the treatment reactor, while the Reactor B consortia only exceeded the treatment reactor yield at a F/M ratio of 2. Only microorgan-

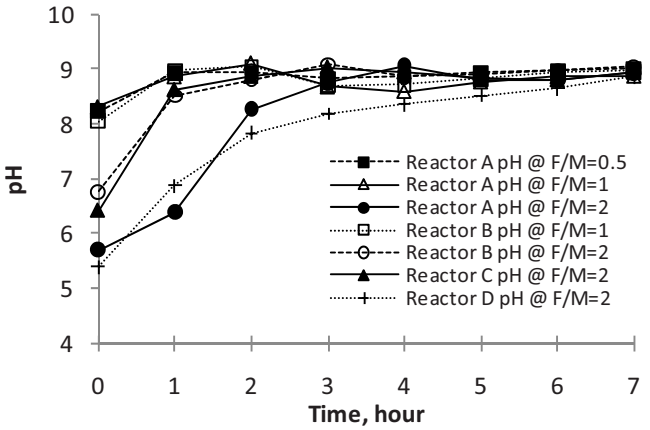

Fig. 4. pH variation over an operational cycle for select sidestream reactors

isms from Reactors C and D exhibited higher polymer yields in sidestream operations than their treatment reactor configurations. These observations suggest that optimal PHA production will not demand that the treatment reactor be supplied excess quantities of substrate, but rather that some of the organic carbon can be conserved for polymer production in the sidestream reactors and the treatment reactor should simply be operated to enrich for microorganisms capable of feast/famine PHA synthesis.

Estimating PHA Production Capacity for a Full-Scale System

This research was centered on answering one core question—is there sufficient organic carbon in municipal wastewater to accomplish PHA production and adequately treat wastewater? To extrapolate the bench-scale results presented and discussed herein to a full-scale SBR facility, we first need to establish how much organic carbon would be available for both processes. As noted, the high value organic carbon substrate required for PHA production would be generated through an in-line primary solids fermenter. Modeling fermenter operation after the results of Bouzas et al. (2007) and applying a fermenter SRT of 8 days, a WWT system receiving an influent flow of $265,000$ L day^{-1} (1 million gallons per day) would yield 665–688 kg of soluble COD per day (fermenter effluent concentration of 162–182-mg soluble COD per L). Note that the influent volatile suspended solid observed by Bouzas et al. (2007), equivalent to 620–770 kg per day for our scenario, would be consistent with moderate strength municipal wastewater (Tchobanoglous et al. 2003). According to Bouzas et al. (2007), this waste stream would be highly enriched in organic acids, which are the critical precursors to PHA synthesis in this waste stream.

For Scenario 1 [PHA production concurrent with treatment, Fig. 2(a)] and considering treatment Reactor A, which produced the most PHA (Table 2), over $2,050$ kg day^{-1} of soluble COD would be required for process success. The fermenter could not generate sufficient carbon to accomplish both treatment and PHA production for this operational scenario. In fact, organic carbon generated in the fermenter would only be sufficient to drive concurrent PHA production and WWT for Reactors C and D. However, for these latter treatment reactor scenarios, greater PHA production was observed in the associated sidestream reactors, which would support implementation of Scenario 2. Aside from the carbon limitation, peak PHA accumulation was observed to occur within an operational cycle and not at the end. Not only would it be a challenge to determine when to harvest the PHA-rich biomass but it is also unclear how this would affect overall process stability. In particular, it could be difficult to maintain a critical mass of feast-famine PHA synthesizing microbes since a potentially large fraction of the necessary population would be removed from the system of each operational cycle.

Considering Scenario 2 [Fig. 2(b)], the treatment SBR operations would be modeled after Reactor D (Table 1). According to the results from this study, the applied OLR in this treatment reactor would be sufficient to enrich for microbes capable of feast/famine PHA synthesis. To achieve the target OLR, approximately 57–85% of the fermenter effluent stream would be directed into the treatment reactor. The remaining fermenter liquor would be fed to the sidestream PHA reactor for polymer production. Assuming a yield of 0.29 kg PHA per kg soluble COD substrate provided (Table 3 for the Reactor D consortium), approximately 11–36 tonne (12–40 tons) of PHA could be produced annually. This PHA yield is in the range of that observed by others on a variety of substrates using mixed microbial consortia [ranging from high purity (pure acetate) to complex (industrial waste), as summarized in Serafim et al. (2008)]. Note that the quantity of biomass wasted daily from the treatment reactor would be sufficient to maintain a F/M ratio of approximately 1, which correlates with the PHA yield value used for these sidestream estimates (Table 3). Regarding commodity value, according to Gurieff and Lant (2007), the current market price for PHA exceeds $10 per kg. Thus, the proposed WWT-PHA production system could generate gross annual revenue of approximately $110,000–$360,000. Polymer processing and purification costs would impact this potential revenue stream, although opportunities exist to produce commodities that significantly minimize PHA processing requirements (Coats et al. 2008). Thus, even the low end of this range could potentially improve WWT operation finances. Moreover, PHA production could be potentially increased with enhanced fermenter optimization and pulse feeding substrate to the sidestream PHA reactor.

Based on the results presented and discussed herein, future investigations focused on advancing this process will need to both extend the bench-scale research to a pilot-scale level and evaluate other reactor operating modes such as plug flow reactors. The pilot-scale investigations will also need to consider the potential impacts on methane production in a municipal WWTF. The proposed process herein converts organic matter in the primary solids to PHA, which potentially conflicts with conventional WWT operations wherein primary solids would be anaerobically converted to energy.

Conclusions

The purpose of the research reported herein was to establish the feasibility of integrating PHA production within municipal WWT. Four bench-scale WWT reactors operated in a sequencing batch mode were tested at decreasing OLRs to assess the potential to enrich for microbes capable of feast/famine PHA synthesis that could concurrently produce high quality treated effluent. For each treatment SBR, sidestream batch reactors receiving elevated quantities of primary solids fermenter liquor were operated to evaluate the ability to produce high quantities of PHA. Key conclusions from this study are as follows:

- A SBR operated to achieve nitrification can concurrently enrich for microorganisms capable of feast-famine PHA synthe-

sis. The process requires in-line fermentation of primary solids to maximize recovery of influent organic carbon and to provide necessary PHA precursors.
- The enriched microbial consortium capable of feast/famine PHA synthesis can be leveraged for PHA production in a side-stream reactor.
- A 265,000 L day^{-1} (1 million gallon per day) WWT-PHA production system could yield approximately 11–36 tonne (12–40 tons) of PHA annually.

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