Production of Polyhydroxyalkanoate During Treatment of Tomato Cannery Wastewater

Hsin-Ying Liu[1], Patrizia V. Hall[2], Jeannie L. Darby[1], Erik R. Coats[3], Peter G. Green[1], Donald E. Thompson[1], Frank J. Loge[1]*

ABSTRACT: Polyhydroxyalkanoate (PHA) production was achieved using tomato cannery waste coupled with a mixed microbial culture during wastewater treatment. The two-stage PHA production process comprised a sequencing batch reactor (SBR), operating under a periodic feast–famine regime, to accomplish simultaneously wastewater treatment and selection of PHA-accumulating microbes, followed by a batch reactor for the production of PHA-rich biomass. The SBRs were efficient at removing soluble carbon (84%), ammonia (100%), and phosphorus (76%). Meanwhile, PHA-accumulating microbes were enriched under the SBR operating conditions, and PHA content on a cell-weight basis was within the range 7 to 11% in nonfiltered wastewater and 2 to 8% in filtered wastewater. Subsequently, batch studies were implemented with varying loading rates, ranging from 0.4 to 3.2 food-to-microorganism ratios. A maximum 20% PHA content on a cell-weight basis was obtained. Based on the experimental results, a PHA biosynthesis-degradation kinetic model was developed to (1) aid in the design of a pilot- or full-scale PHA production process coupled with wastewater treatment and (2) determine optimal conditions for harvest of PHA-rich biomass. *Water Environ. Res.,* **80,** 367 (2008).

KEYWORDS: polyhydroxyalkanoates, wastewater treatment, tomato cannery wastewater.

doi:10.2175/106143007X221535

Introduction

Polyhydroxyalkanoates (PHAs) are biodegradable thermoplastics that are synthesized from bacteria metabolizing renewable organic carbon sources. The PHAs have recently gained recognition as a substitute for conventional petroleum-based plastics. Relative to conventional petroleum-based plastics, PHAs have a lower environmental effect and reduced reliance on nonrenewable fossil fuels (Akiyama et al., 2003). Currently, commercial PHA production typically uses a pure microbial culture, such as *Ralstonia eutropha*, grown on a synthetically derived carbon substrate, such as corn or soybean oil. However, the high costs related to feedstock production and aseptic reactor operation limit the potential replacement of petroleum-based thermoplastics with PHAs (Lee, 1996). The use of inexpensive substrates (i.e., crude carbon substrates from industrial food or agricultural wastes) and mixed cultures (i.e., activated sludge) have been proposed (Braunegg et al., 2004; Reis et al., 2003; Salehizadeh and Van Loosdrecht, 2004) as methods to mitigate approximately 50% of the costs associated with carbon substrate synthesis and 30 to 40% of the costs associated with aseptic operating conditions (Akiyama et al., 2003; Braunegg et al., 2004). Some cost-saving approaches have been conducted. For example, Hassan et al. (2002) incorporated PHA production from *Ralstonia eutropha* fed palm oil mill effluent. Dionisi et al. (2001) used activated-sludge-fed acetic, lactic, and propionic acids for PHA production. Most recently, Punrattanasin et al. (2006) used activated-sludge-fed, high-acetic-acid industrial wastewater, showing the potential of PHA production using industrial wastewaters as the substrate. In this study, PHA production has been achieved using an actual industrial waste coupled with a mixed microbial culture under nonaseptic conditions. In addition, this study integrated the perspective of PHA production into an actual wastewater treatment process (i.e., concurrent PHA production and satisfactory effluent quality), thus encompassing industrial wastewater treatment, waste reduction, and resource regeneration (i.e., waste sludge to PHA-rich biomass), all within one system.

Waste from industrial food processing was chosen as the feedstock for this study for two reasons. The readily biodegradable, high-organic-content composition of industrial food processing waste streams meets criteria for PHA production (Daigger and Grady, 1982). Further, secondary treatment of industrial-food-processing waste streams alleviates many potential environmental concerns associated with current land-application practices. In the United States, most food processors discharge their untreated or partially treated wastewater to fields, assuming that it is similar to an irrigation supply or soil amendment. However, problems can arise from this practice, as overapplication of such wastewater can lead to nuisance conditions and adverse ground water effects (U.S. EPA, 1999).

In most past studies, the process of biosynthetic PHA production has been accomplished in two sequential stages (Chua et al., 2003; Majone et al., 2006). In the first stage, selective pressures are imposed on a microbial culture to enrich for microorganisms capable of accumulating PHA. Selective pressures have included the use of the following:

(1) Nitrogen (Ma et al., 2000) or phosphorus-limited (Rhu et al., 2003) feedstocks,
(2) A cyclic feast–famine feeding pattern (Serafim et al., 2004), and
(3) A microaerophilic–oxic (Satoh et al., 1998) or anoxic–oxic (Chua et al., 2003) cycling over the course of a single feeding period.

In the second stage, the enriched culture is fed a high concentration carbon feedstock composed of PHA precursor metabolites (i.e., short-chain volatile fatty acids). One method of

[1] Department of Civil and Environmental Engineering, University of California, Davis.

[2] CH2M HILL, Los Angeles, California.

[3] Department of Civil Engineering, University of Idaho, Moscow.

* Department of Civil and Environmental Engineering, University of California, Davis, One Shields Avenue, Davis, CA 95616; e-mail: fjloge@ucdavis.edu.

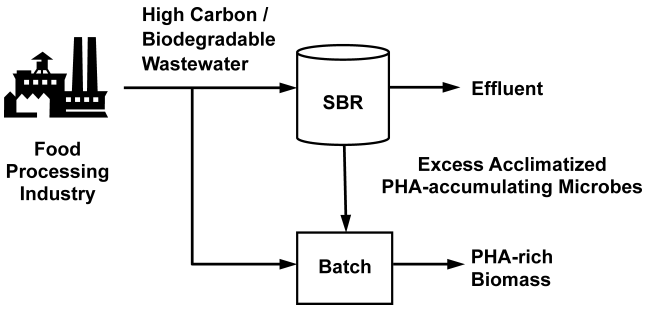

Figure 1—Proposed PHA production process during wastewater treatment.

obtaining a feedstock rich in PHA precursor metabolites is through the prior (i.e., before using as a PHA production feedstock) fermentation of a particular waste stream (Dionisi et al., 2004; Takabatake et al., 2000).

In this study, a two-stage process, as shown in Figure 1, was used for PHA production concurrent with wastewater treatment. Raw tomato cannery wastewater was provided directly to both the first and second stages, represented in Figure 1 as the sequencing batch reactor (SBR) and batch reactor, respectively. The SBR was operated in a cyclic feast–famine feeding pattern (Salehizadeh and Van Loosdrecht, 2004), to elicit selection of microorganisms capable of producing PHA concurrent with the removal (i.e., treatment) of organic material (Dionisi et al., 2001). The batch reactor was fed raw tomato cannery wastewater at a food-to-microorganism (F/M) greater than in the SBR, to elicit the production of large quantities of intracellular PHA.

The overall goal of this study was to evaluate the feasibility of producing PHA, while simultaneously treating tomato cannery wastewater. The specific objectives were to

(1) Evaluate the efficacy of using a SBR to concurrently select microorganisms capable of producing PHA, while treating tomato cannery wastewater;
(2) Determine the effects of varied substrate loads (i.e., F/M ratio) on PHA production; and
(3) Provide a kinetic model of PHA synthesis and degradation, to predict PHA content on a cell-weight basis over time.

Methods

Feedstocks and Microbial Consortium. Wastewater from a tomato cannery in Woodland, California, was collected on a weekly basis during the cannery season and transported back to the laboratories in the Department of Civil and Environmental Engineering at the University of California, Davis (UCD). The wastewater was immediately tested for soluble chemical oxygen demand (sCOD), orthophosphate, total phosphorous (TP), total Kjeldahl nitrogen (TKN), and total suspended solids (TSS) and subsequently refrigerated at 4°C, pending further experiments. A fraction of the tomato cannery wastewater was additionally filtered through a coarse sieve (standard test sieve, 38 μm, W.S. Tyler Company, Mentor, Ohio) before storage. The microbial consortium used to initially start up the SBRs was obtained as activated sludge from the UCD wastewater treatment facility.

Sequencing Batch Reactor Operation. Two SBRs with a 4-L working volume were initially inoculated with activated sludge obtained from the UCD wastewater treatment plant. The two reactors were then separately fed, with a 0.25 day^{-1} dilution rate, nonfiltered and filtered tomato cannery wastewater. The reactors were continuously operated on a 24-hour cycle under fully oxic conditions at a temperature of 20 ± 1°C. A settling stage was not implemented before removing liquid to maintain a 0.25-day^{-1} dilution rate. Hence, 1 L of completely mixed liquid, containing both microorganisms and treated cannery wastewater, was wasted daily to maintain a 4-day hydraulic retention time, which, in this case, was equivalent to the solids retention time (SRT). Aeration was maintained through a 23-cm- (9-in.-) diameter Sanitaire Silver Series II membrane fine-bubble disc diffuser (Sanitaire, Brown Deer, Wisconsin) installed at the base of the reactor. The reactors were operated for 12 days (equivalent to 3 SRTs) before sampling, to ensure steady-state conditions (Tchobanoglous et al., 2003).

Batch Reactor Operation. For PHA production, biomass was withdrawn from an SBR and evenly seeded into four independent 2-L batch reactors. Defined volumes of filtered tomato cannery wastewater were added to each 2-L batch reactor, to achieve F/M ratios of 0.4, 0.6, 1, and 2 (F/M ratios were calculated as the COD [expressed as mg/L] divided by the mixed liquor suspended solids [MLSS] concentration [expressed as mg/L], both reflecting values in the reactor immediately after feeding). Each batch reactor was operated under fully oxic conditions for a 24-hour period. Two additional duplicate individual batch reactors were operated at an F/M ratio of 3.2, to obtain data for the PHA synthesis–degradation model.

Analytical Techniques. Soluble carbon use was measured by the COD test, in accordance with method 5220-D of *Standard Methods* (APHA et al., 1998), using Hach high-range ampoules (Hach Company, Loveland, Colorado). Soluble orthophosphate and total phosphorous were determined using Hach methods 8048 and 8190, respectively. The Hach methods are equivalent to method 4500-PE of *Standard Methods* (APHA et al., 1998). Ammonia was measured using a continuous-flow Timberline model 383 inorganic nitrogen analyzer (Timberline Instruments Inc., Boulder, Colorado). Samples for soluble constituents were filtered through 0.22-μm filters before analyses (Millipore Corporation, Billerica, Massachuetts). The MLSS and mixed liquor volatile suspended solids (MLVSS) were determined gravimetrically, in accordance with method 2540-D of *Standard Methods* (APHA et al., 1998).

The PHA-rich biomass from all of the wastewater-based processes was chlorinated with 10% sodium hypochlorite for 5 minutes and centrifuged at 10 000 × g for 10 minutes, to obtain a pellet. The pellet was resuspended in deionized water and dried at 60°C for 4 hours. The PHA content of the biomass was determined using methanolysis and gas chromatography/mass spectroscopy (GC-MS, model 6890-5973N, Agilent Technologies, Palo Alto, California), following the method of 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 deionized water, the organic layer was recovered for analysis. The identical digestion process was used for the PHA polymer standards poly(3-hydroxybutyric acid) and poly(3-hydroxybutyric acid-*co*-3-hydroxyvaleric acid) (Fluka and Aldrich, Sigma-Aldrich, St. Louis, Missouri). The GC-MS was performed with split injection under an initial oven temperature of 40°C (2 minutes) ramped up to 200°C at 5°C/min using a 30-m ZB-624 column (0.25 mm internal diameter, 1.4-μm film; Phenomenex, Torrance, California). The compounds (methyl ester derivatives) were scanned by comparing the mass spectroscopy spectra in the 275k MS library to confirm PHA forms. The specified PHA polymers were identified by retention time and mass

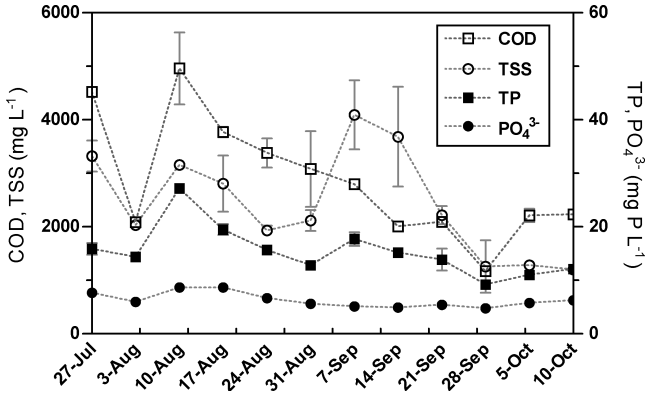

Figure 2—Selected measures of water quality of untreated tomato cannery wastewater during 2005 canning season. Error bars represent 95% confidence intervals (n = 3).

spectral matching 3-hydroxybutyric acid (3HB) and 3-hydroxyvaleric acid (3HV) and quantified based on the external and internal standards. Total cellular PHA content was determined on a percent-weight-cell basis (i.e., mass of PHA/mass of biomass, w/w).

Results and Discussion

Characterization of Feedstock. The soluble carbon and nutrient concentrations in the tomato cannery waste varied temporally, as a result of the processing schedule of the cannery (Figure 2), because various tomato products are processed at different times over the course of the season. The average COD concentration in the tomato cannery wastewater was approximately 3000 mg/L. As a basis of comparison, a typical value for municipal wastewater ranges between 260 and 900 mg/L (Tchobanoglous et al., 2003). The average concentrations of orthophosphate, total phosphorus, and TKN in the cannery wastewater were approximately 6.3 mg-P/L, 15.5 mg-P/L, and 120 mg-N/L, respectively. The concentration of total phosphorus in the tomato cannery wastewater was slightly above the range 4 to 12 mg-P/L estimated for a typical municipal wastewater, while the TKN concentration was within the typical range 20 to 705 mg-N/L (Tchobanoglous et al., 2003). Additionally, TSS concentrations were approximately 2500 mg/L on average; typical values for untreated domestic wastewater are 120 to 400 mg/L (Tchobanoglous et al., 2003). In summary, the carbon content in the tomato cannery wastewater was found to be at least three times higher than in typical municipal wastewater, whereas phosphorous and nitrogen levels were found to be approximately the same or slightly greater. These nutrient loads led to the hypothesis that the tomato cannery waste stream contains excess carbon relative to other macronutrients, which, in turn, may result in imbalanced growth conditions facilitating PHA production.

Sequencing Batch Reactor Operation for Simultaneous Wastewater Treatment and Production of Polyhydroxyalkanoate. Nonfiltered and filtered tomato cannery wastewater was treated in parallel SBRs subject to periodic feeding under fully oxic conditions, with the objective of selecting microorganisms capable of producing PHA concurrent with wastewater treatment. For the filtered wastewater, the larger solids in raw tomato cannery wastewater were removed through a coarse sieve (38 μm), to simulate the function of primary sedimentation.

The water-quality patterns indicate that the parallel operating SBRs have similar efficacy in treating nonfiltered and filtered

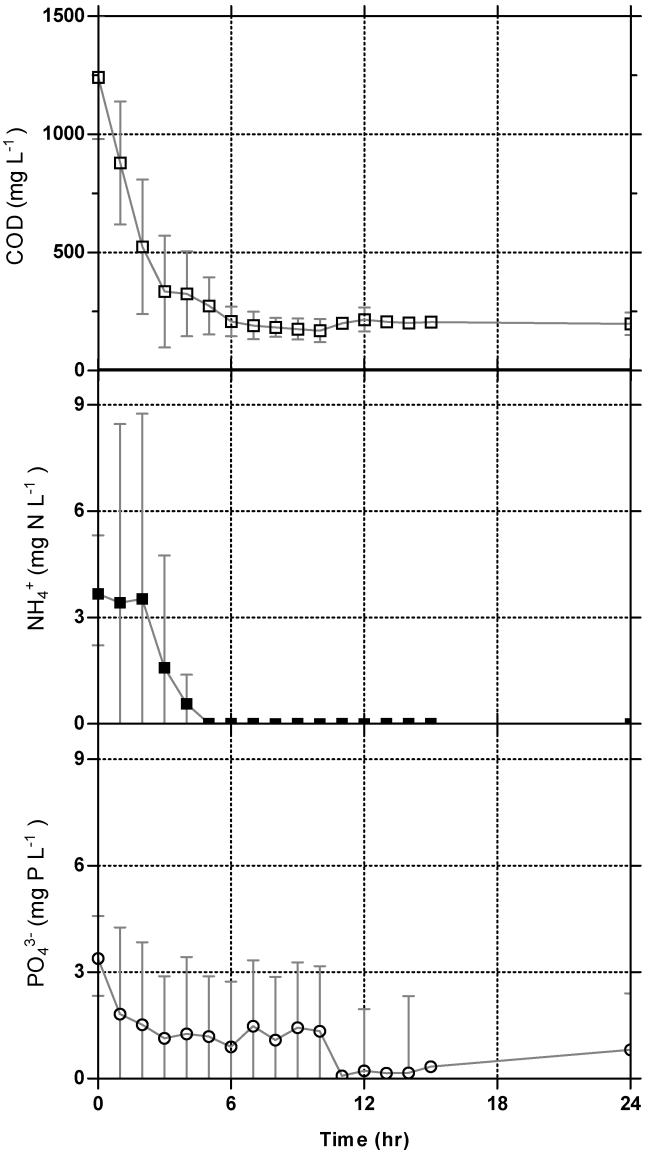

Figure 3—Concentration of soluble COD, nitrogen, and phosphorous over the course of one feeding cycle in the SBR. Error bars represent 95% confidence intervals (n = 5).

tomato waste. Following stabilization, soluble carbon and nutrient amounts for nonfiltered tomato cannery waste were determined during the feed and reaction stages over 24 hours. The carbon and nutrient reduction capacity of the SBR was then determined, by averaging the data for each time point across all five sampling cycles, as shown in Figure 3; COD was reduced by 84%, ammonia by 100%, and orthophosphate by 76%. Despite varied influent concentrations for both nonfiltered and filtered tomato cannery wastewater, the SBRs were efficient at removing soluble carbon, nitrogen, and phosphorous. Within 5 hours after feeding, COD levels in both SBRs dropped significantly, to a steady-state concentration of approximately 200 mg/L, whereas ammonia levels were nondetectable (detection limit = 1 mg-N/L) for both reactors, and orthophosphate values were roughly 0.50 mg-P/L for nonfiltered wastewater and 2.29 mg-P/L for filtered wastewater, respectively.

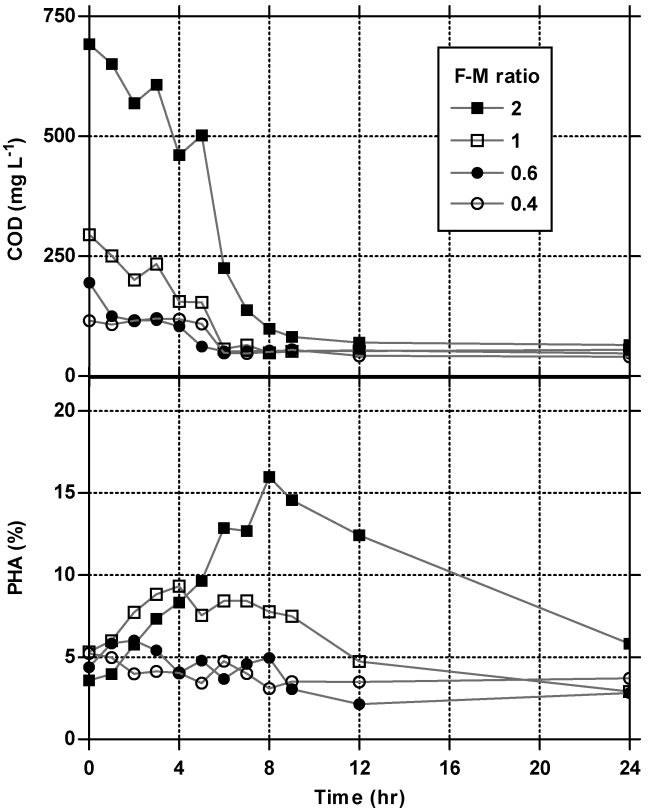

Figure 4—Comparison of soluble COD and biomass PHA within batch reactors operated with varied F/M ratios (2, 1, 0.6, and 0.4) of filtered tomato cannery wastewater. Data is depicted over one feeding cycle.

The activated sludge in the SBR reactor was exposed to a periodic influx of substrate (i.e., COD) availability (i.e., feast stage during the first 5 hours), followed by unavailability (i.e., famine stage during the remaining reaction phase). This cyclic feast–famine feeding pattern resulted in a microbial consortium capable of producing 7 to 11% PHA on a dry-weight-cell basis [assessed as poly-3-hydroxybutyrate, P(3HB)] in nonfiltered wastewater and 2 to 8% PHA in filtered wastewater.

Effect of Food-to-Microorganism Ratios on Chemical Oxygen Demand Removal and Polyhydroxyalkanoate Production. Results from the batch reactors were used to

(1) Assess the feasibility of providing high substrate loads to increase the cell mass concentration of PHA, and
(2) Evaluate the effect of the F/M ratio on PHA production.

In all four batch reactors (representing F/M ratios of 0.4, 0.6, 1, and 2), PHA synthesis began immediately after carbon substrate addition and continued throughout the feast phase (Figure 4). Once the soluble substrate concentration was depleted, the cells entered a famine phase, and the intracellular PHA concentration was rapidly consumed (Figure 4). The patterns of PHA consumption, PHA degradation, and COD removal apparent in a cyclic feast–famine feeding schedule were more pronounced at higher F/M ratios. High substrate loads led to higher COD consumption rates and longer feast periods, during which time, PHA was continuously produced. The positive correlation between substrate loads and PHA production agrees with a previous study of carbon loading effects (8.5 to 20 g COD/L) on intracellular PHA concentrations (Dionisi et al., 2006).

The trigger for biological storage-response metabolism associated with substrate concentration gradation can also explain PHA enrichment at relatively high F/M ratios. Under transient conditions of increasing soluble carbon substrate (i.e., increasing F/M ratio), the physiological response of a mixed microbial consortium can increasingly manifest itself as a carbon and energy storage response (instead of growth response), only if the carbon substrate can be readily transported into the microbial cell and degraded to the storage polymer precursors (Daigger and Grady, 1982). Because the tomato cannery wastewater is readily biodegradable, as shown in this study, when coupled with a high substrate-driving force permitting rapid absorption of the soluble organics (Tchobanoglous et al., 2003), it complements the rapid transportation conditions. This means that PHA storage corresponds to relatively high F/M ratios in the range 0.4 to 2.

Microorganisms grown in a nitrogen-deficient environment tend to produce more intracellular fatty material or carbohydrates (i.e., PHA, in this case), resulting in a smaller proportion of nitrogen in the empirical cell formulas (Rittmann and McCarty, 2001). Thus, both feast–famine selective pressure (i.e., operation of SBRs) and the nature of the tomato wastewater (i.e., highly biodegradable carbon with relatively low nutrient content) creates growth-limiting conditions, which facilitate PHA production.

Compared with the treatment of filtered cannery wastewater, the four batch reactors receiving nonfiltered wastewater had comparable carbon and nutrient removal conditions, but lower PHA content and an irregular PHA synthesis–degradation pattern. The same phenomenon was noted in the parent SBRs, suggesting that the tomato wastewater solids did not hinder biodegradation in the system, but that nonfiltered solids introduced a bias in the MLSS mass. This bias led to both an underestimation and severe variability within samples when calculating PHA content on a cell-weight basis. In addition, tomato wastewater solids may interfere with the extraction and purification step when harvesting the PHA or result in a waste residual stream that is more difficult to treat or dispose of after the extraction. Therefore, primary sedimentation is recommended to achieve reliable PHA production.

Under current California regulations, waste streams from food processing industries do not require treatment, unless discharged to state waterways. Most facilities choose to use the wastewater to irrigate lands. In the future, such practices are likely to fall under increased regulatory scrutiny, resulting in stringent treatment requirements. Moreover, there is a cost associated with irrigation. Conversely, integrating the commodity of PHA production within a waste-management program would conceptually enable food-processing industries to realize additional income from converted waste.

Kinetic Model of Polyhydroxyalkanoate Synthesis and Degradation on Tomato Cannery Wastewater. A model of PHA synthesis and degradation was developed to predict PHA content on a cell-weight basis over time. Two individual batch reactors, acting as duplicates, were adjusted to an F/M ratio of 3.2 and operated under oxic conditions similar to previous experiments. The model was fit to data collected in these batch reactors to

(1) Obtain specific values of the kinetic rate coefficients, and
(2) Assess the fit of the model to empirical data.

Assuming that composite PHA synthesis and degradation correlates with the main effect of biomass concentration and

substrate–biomass interaction, an observation that is based on the shape of the PHA synthesis–degradation curve, the rate of PHA synthesis–degradation can be described as follows:

$$r_{PHA} = X_v(k_1 C - k_2) \quad (1)$$

Where

C = substrate concentration at time t (COD, mg/L),
X_v = biomass concentration (MLSS, mg/L),
k_1 = substrate–biomass interaction coefficient for PHA production (L/mg · h), and
k_2 = biomass coefficient for PHA degradation (hour^{-1}).

Additionally, the experimental substrate removal rate was determined using zero-order kinetics, with respect to COD, as follows:

$$C = C_0 - k_c t \quad (2)$$

Where

C_0 = influent substrate concentration (COD, mg/L),
k_c = kinetic constant for substrate degradation (mg/L · h), and
t = reaction time (hours).

Substitution of eq 2 into eq 1 yields the following expression for PHA synthesis–degradation:

$$\frac{dPHA}{dt} = r_{PHA} = X_v(k_1(C_0 - k_c t) - k_2) \quad (3)$$

Therefore, PHA synthesis–degradation can be obtained by the integration of eq 3, as follows:

$$\int \frac{dPHA}{X_v} = \int_0^t (k_1 C_o - k_c k_1 t - k_2)dt$$

$$\frac{PHA}{X_v} = k_1 C_0 t - \frac{k_c k_1 t^2}{2} - k_2 t \quad (4)$$

Because the biomass concentration can be assumed to be constant in an equilibrated system, the PHA content on a cell-weight basis over time can be predicted according to eq 5, as follows:

$$\text{PHA content } (\%) = k_1 C_0 t - \frac{k_c k_1 t^2}{2} - k_2 t \quad (5)$$

Fitting the batch reactor data to this model yields the following coefficients: k_1 = 0.0059 L/mg · h, k_2 = 0.1068 hour^{-1}, and k_c = 38.4820 mg/L · h. Figure 5 illustrates COD removal and PHA accumulation and degradation data derived from the duplicate batch reactors in relation to the kinetic model. The kinetic model adequately fit the empirical data. Equation 5 demonstrates the link between PHA production and substrate loads (i.e., initial COD) and additionally provides information for the optimal time to harvest PHA-rich biomass during the enrichment process. Recognizing that the values of k_1, k_c, and k_2 will be wastewater-specific, the model also provides a basis for designing and operating PHA production processes for pilot- or full-scale wastewater treatment systems.

Conclusions

Concurrent tomato wastewater treatment (i.e., 84% COD removal, 100% ammonia removal, and 76% orthophosphate removal) and PHA production (i.e., 20% PHA content on a cell-weight basis) is realistic. The yield of PHA is influenced by the F/M ratio, with higher ratios eliciting higher quantities of PHA within cellular biomass. A kinetic model was developed that adequately described PHA biosynthesis and degradation. The model can be used (1) in the design of a pilot- or full-scale PHA production process coupled with wastewater treatment, and (2) to determine optimal conditions for harvest of PHA-rich biomass.

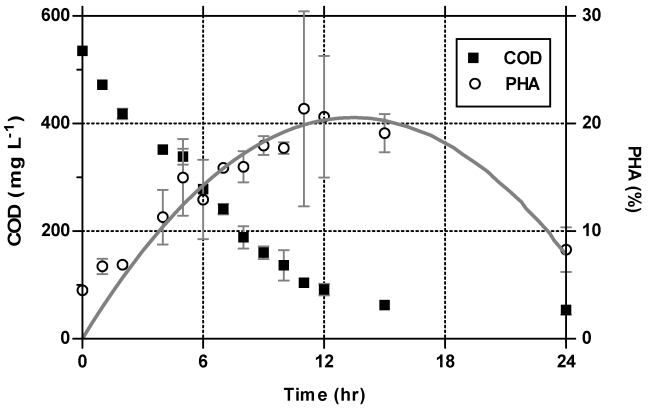

Figure 5—Fit of PHA synthesis–degradation model (eq 5) to experimental data. Error bars represent 95% confidence intervals.

Submitted for publication February 6, 2007; revised manuscript submitted September 12, 2007; accepted for publication November 5, 2007.

The deadline to submit Discussions of this paper is July 15, 2008.

References

Akiyama, M.; Tsuge, T.; Doi, Y. (2003) Environmental Life Cycle Comparison of Polyhydroxyalkanoates Produced from Renewable Carbon Resources by Bacterial Fermentation. *Polym. Degrad. Stab.,* **80** (1), 183–194.

American Public Health Association; American Water Works Association; Water Environment Federation (1998) *Standard Methods for the Examination of Water and Wastewater,* 19th ed.; American Public Health Association: Washington, D.C.

Braunegg, G.; Bona, R.; Koller, M. (2004) Sustainable Polymer Production. *Polym-Plast. Technol. Eng.,* **43** (6), 1779–1793.

Braunegg, G.; Sonnleitner, B.; Lafferty, R. M. (1978) Rapid Gas-Chromatographic Method for Determination of Poly-Beta-Hydroxybutyric Acid in Microbial Biomass. *Euro. J. Appl. Microbiol. Biotechnol.,* **6** (1), 29–37.

Chua, A. S. M.; Takabatake, H.; Satoh, H.; Mino, T. (2003) 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** (15), 3602–3611.

Daigger, G. T.; Grady, C. P. L. (1982) The Dynamics of Microbial-Growth on Soluble Substrates—A Unifying Theory. *Water Res.,* **16** (4), 365–382.

Dionisi, D.; Majone, M.; Papa, V.; Beccari, M. (2004) Biodegradable Polymers from Organic Acids by Using Activated Sludge Enriched by Aerobic Periodic Feeding. *Biotechnol. Bioeng.,* **85** (6), 569–579.

Dionisi, D.; Majone, M.; Tandoi, V.; Beccari, M. (2001) Sequencing Batch Reactor: Influence of Periodic Operation on Performance of Activated Sludges in Biological Wastewater Treatment. *Ind. Eng. Chem. Res.,* **40** (23), 5110–5119.

Dionisi, D.; Majone, M.; Vallini, G.; Di Gregorio, S.; Beccari, M. (2006) Effect of the Applied Organic Load Rate on Biodegradable Polymer Production by Mixed Microbial Cultures in a Sequencing Batch Reactor. *Biotechnol. Bioeng.,* **93** (1), 76–88.

Hassan, M. A.; Nawata, O.; Shirai, Y.; Rahman, N. A. A.; Yee, P. L.; Bin Ariff, A.; Ismail, M.; Karim, A. (2002) A Proposal for Zero Emission from Palm Oil Industry Incorporating the Production of

Polyhydroxyalkanoates from Palm Oil Mill Effluent. *J. Chem. Eng. Japan,* **35** (1), 9–14.

Lee, S. Y. (1996) Plastic Bacteria? Progress and Prospects for Polyhydroxyalkanoate Production in Bacteria. *Trends Biotechnol.,* **14** (11), 431–438.

Ma, C. K.; Chua, H.; Yu, P. H. F.; Hong, K. (2000) Optimal Production of Polyhydroxyalkanoates in Activated Sludge Biomass. *Appl. Biochem. Biotechnol.,* **84/86,** 981–989.

Majone, M.; Beccari, M.; Di Gregorio, S.; Dionisi, D.; Vallini, G. (2006) Enrichment of Activated Sludge in a Sequencing Batch Reactor for Polyhydroxyalkanoate Production. *Water Sci. Technol.,* **54** (1), 119–128.

Punrattanasin, W.; Randall, A. A.; Randall, C. W. (2006) Aerobic Production of Activated Sludge Polyhydroxyalkanoates from Nutrient Deficient Wastewaters. *Water Sci. Technol.,* **54** (8), 1–8.

Reis, M. A. M.; Serafim, L. S.; Lemos, P. C.; Ramos, A. M.; Aguiar, F. R.; Van Loosdrecht, M. C. M. (2003) Production of Polyhydroxyalkanoates by Mixed Microbial Cultures. *Bioprocess. Biosyst. Eng.,* **25** (6), 377–385.

Rhu, D. H.; Lee, W. H.; Kim, J. Y.; Choi, E. (2003) Polyhydroxyalkanoate (PHA) Production from Waste. *Water Sci. Technol.,* **48** (8), 221–228.

Rittmann, B. E., McCarty, P. L. (2001) *Environmental Biotechnology: Principles and Applications;* McGraw Hill: New York, 128–129.

Salehizadeh, H.; Van Loosdrecht, M. C. M. (2004) Production of Polyhydroxyalkanoates by Mixed Culture: Recent Trends and Biotechnological Importance. *Biotechnol. Adv.,* **22** (3), 261–279.

Satoh, H.; Iwamoto, Y.; Mino, T.; Matsuo, T. (1998) Activated Sludge as a Possible Source of Biodegradable Plastic. *Water Sci. Technol.,* **38** (2), 103–109.

Serafim, L. S.; Lemos, P. C.; Oliveira, R.; Reis, M. A. M. (2004) Optimization of Polyhydroxybutyrate Production by Mixed Cultures Submitted to Aerobic Dynamic Feeding Conditions. *Biotechnol. Bioeng.,* **87** (2), 145–160.

Takabatake, H.; Satoh, H.; Mino, T.; Matsuo, T. (2000) Recovery of Biodegradable Plastics from Activated Sludge Process. *Water Sci. Technol.,* **42** (3–4), 351–356.

Tchobanoglous, G.; Burton, F. L.; Stensel, H. D. (2003) *Wastewater Engineering, Treatment and Reuse;* Metcalf and Eddy Inc.: New York.

U.S. Environmental Protection Agency (1999) Multimedia Environmental Compliance Guide for Food Processors. U.S. Environmental Protection Agency: Washington, D.C.