Bioresource Technology 101 (2010) 1049–1053

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Production of polyhydroxybutyrate by activated sludge performing enhanced biological phosphorus removal

Michael Rodgers, Guangxue Wu *

Civil Engineering, College of Engineering and Informatics, National University of Ireland, Galway, Ireland

ARTICLE INFO

Article history:
Received 12 June 2009
Received in revised form 28 August 2009
Accepted 28 August 2009
Available online 17 September 2009

Keywords:
Activated sludge
Biodegradable plastics
Enhanced biological phosphorus removal
Polyhydroxybutyrate

ABSTRACT

In this study, polyhydroxybutyrate (PHB) – a biodegradable plastics material – was produced by activated sludge performing enhanced biological phosphorus removal (EBPR) in batch experiments under anaerobic, aerobic and anaerobic/aerobic conditions. Under anaerobic conditions, the maximum PHB content of the dry biomass was 28.8% by weight, while under aerobic or anaerobic/aerobic conditions, the maximum PHB content was about 50%. The PHB production rate with respect to the volatile suspended solids (VSS) was: (i) 70 mg/(g VSS) h under aerobic conditions that followed anaerobic conditions, (ii) 156 mg/(g VSS) h under anaerobic condition, and (iii) 200 mg/(g VSS) h under aerobic conditions with energy also supplied from polyphosphate. A side stream, with initially anaerobic conditions for PHB accumulation and phosphorus release, and then aerobic conditions for PHB accumulation, was proposed. In this side stream, biomass with a high PHB content and a high PHB production rate could be both achieved.

1. Introduction

In wastewater treatment processes, storage of organic carbon can be considered as one type of survival mechanism for microorganisms experiencing dynamic "feast" and "famine" conditions (Daigger and Grady, 1982; van Loosdrecht et al., 1997). In these processes, polymer-accumulating organisms can be enriched, and two important carbon polymers, polyhydroxyalkanoate (PHA) and glycogen, are usually stored (Dircks et al., 2001). When acetate and propionate/glucose are the carbon substrates, polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV) are the main PHAs, respectively (Hood and Randall, 2001).

PHA plays an important role in wastewater treatment processes, and it can be sustainably recovered for the production of biodegradable plastics (Satoh et al., 1998); simultaneously, sludge reduction can be achieved. As a consequence, the costs for PHA production and sludge disposal will be decreased. Different wastewater treatment processes (aerobic dynamic substrate feeding processes and anaerobic/aerobic processes) have been investigated for the accumulation of PHA (Satoh et al., 1998; Takabatake et al., 2000; Serafim et al., 2004). An aerobic dynamic substrate feeding process can be optimized for PHA accumulation and is suitable for treating industrial wastewater with excess organic carbon but with limited nitrogen and phosphorus. Anaerobic and aerobic alternating processes, such as enhanced biological phosphorus removal (EBPR), on the other hand, would be a better choice for treating wastewater with high carbon and phosphorus concentrations. In the EBPR process, polyphosphate-accumulating organisms (PAOs) can be acclimated to simultaneously accumulate PHA and polyphosphate for potential recovery, e.g., PAOs can store polyphosphate up to 15% for possible use as a fertilizer. Many studies have been carried out to examine the function and composition of PHA by EBPR microbial communities (Lemos et al., 1998; Pijuan et al., 2009). However, only limited studies have been carried out on the PHA accumulation in EBPR processes for the purpose of recovery (Satoh et al., 1998; Takabatake et al., 2000; Perez-Feito and Noguera, 2006; Kasemsap and Wantawin, 2007). Among these studies on the PHA recovery from EBPR, some only focused on PHA accumulation inside the treatment reactors (Perez-Feito and Noguera, 2006), while others focused on PHA accumulation potential (Takabatake et al., 2000). It is important to examine the metabolic processes of PAOs by including the dynamics of various polymers so as to optimize the system for both wastewater treatment and resource recovery.

PAOs can accumulate PHA under anaerobic conditions with the energy supplied from polyphosphate degradation or under aerobic conditions with excess external organic carbon being available (Ahn et al., 2007). In this study, PHB production potential by activated sludge performing EBPR under anaerobic, aerobic and anaerobic/aerobic (initially anaerobic and then aerobic) conditions was investigated in batch experiments. Only PHB was focused on because acetate was used as the main carbon substrate and acetate

* Corresponding author. Tel.: +353 91 493084; fax: +353 91 494507.
E-mail addresses: Guangxue.Wu@nuigalway.ie, wuguangxue@tsinghua.org.cn (G. Wu).

0960-8524/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2009.08.107

is mainly stored as PHB (Hood and Randall, 2001). Furthermore, a strategy was proposed for both resource recovery and wastewater treatment.

2. Methods

2.1. EBPR acclimation

A sequencing batch reactor (SBR) was operated at 20 °C and the reactor had a working volume of 5.4 l. The SBR had four cycles per day and each cycle comprised the following phases: fill (15 min), anaerobic (105 min), aerobic (180 min), settle (40 min) and draw/idle (20 min). In each cycle, 1.8 l of treated wastewater were exchanged with a new batch of synthetic wastewater. The reactor was constantly stirred with a magnetic stirrer at 500 rpm during the fill, anaerobic and aerobic phases; during the aerobic phase, air was supplied with an air diffuser located at the bottom of the reactor. Once a day, 750 ml of mixed liquor was withdrawn from the reactor just before the end of the aerobic phase, resulting in a solids retention time (SRT) of 7.2 days if no solids loss occurred during the settle phase.

The components of the synthetic wastewater were: 750 mg/l sodium acetate, 18 mg/l yeast extract, 120 mg/l NH_4Cl, 200 mg/l K_2HPO_4, 130 mg/l $MgSO_4{\cdot}7H_2O$ and 18 mg/l $CaCl_2{\cdot}6H_2O$. Trace elements (1 ml) were added following Barat et al. (2008). Concentrated sulfuric acid (1.4 drops in 1 l) was added to the synthetic wastewater to adjust pH to approximately 6.8. The reactor was seeded with activated sludge taken from Tuam Wastewater Treatment Plant, Co. Galway, Ireland.

2.2. Batch experiments

Batch experiments were carried out to examine PHB accumulation potential under (i) anaerobic, (ii) aerobic, and (iii) anaerobic/aerobic conditions. Seven hundred and fifty milliliter of activated sludge mixed liquor was withdrawn from the SBR and used in each batch experiment.

For the anaerobic PHB accumulation, the 750 ml mixed liquor was settled in a glass flask for 20 min. Then 250 ml of the supernatant was removed. The remaining 500 ml mixed liquor was purged with argon gas to remove oxygen, and 250 ml of synthetic wastewater was added to again provide a total mixed liquor volume of 750 ml. The 250 ml of synthetic wastewater had the same composition as that fed into the parent SBR, except that the sodium acetate concentration was increased to around 2865 mg/l. In addition, the synthetic wastewater was also purged with argon gas to remove oxygen before adding to the glass flask. The glass flask with the 750 ml mixed liquor volume was sealed with a rubber stopper and stirred at 300 rpm in an incubator at 20 °C. Samples were taken at intervals to test soluble and particulate components including: orthophosphate (PO_4–P), sodium acetate, carbohydrate and PHB. The top of the glass flask was supplied with argon gas during sampling so as to maintain anaerobic conditions. The pH ranged from 7.3 to 7.5 during this anaerobic batch experiment.

For the aerobic PHB accumulation, the 750 ml mixed liquor was withdrawn from the SBR and then transferred to a 1000 ml glass flask. The glass flask was placed in an incubator at 20 °C. Air was continuously supplied at the rate of 2 l/min and the dissolved oxygen (DO) concentration was above 4 mg/l during the whole experimental period. Only sodium acetate was added at the start of aeration (2539 mg/l after addition) and after 120 min (2296 mg/l after addition). With the addition of only acetate, nitrogen limitation occurred and this benefited PHB accumulation (Ntaikou et al., 2009). Samples were taken at intervals to test soluble and particulate components including: PO_4–P, sodium acetate, carbohydrate and PHB. The initial pH was 7.6 and this increased to 9.4 by the end of the aerobic batch experiment.

For the anaerobic/aerobic PHB accumulation at 20 °C, the procedure combining the anaerobic PHB accumulation and the aerobic PHB accumulation was used. Firstly, the anaerobic PHB accumulation was carried out as described previously. After the anaerobic PHB accumulation, the mixed liquor was settled for 20 min, and the supernatant liquid (approximately 1/3 of the total volume) was exchanged with the effluent from the SBR to remove the released phosphorus and other nutrients. These settle and exchange processes (washing) were carried out twice. After dilution, nitrogen limitation occurred in the mixed liquor, benefiting PHB accumulation. Then the mixed liquor (around 700 ml) was aerated for PHB accumulation with only excess sodium acetate (2160 mg/l after addition) added at the start of the aeration. Samples were taken at intervals to test soluble and particulate components. The pH ranged from 7.4 to 7.5 during the initial anaerobic batch experiment; then increased to 7.77 after washing; and finally increased to 9.5 by the end of the aerobic batch experiment.

2.3. Analytical methods

Ammonium (NH_4–N), nitrite (NO_2–N), nitrate (NO_3–N) and PO_4–P were analyzed using a Konelab 20 analyzer (Thermo Clinical Labsystems, Vantaa, Finland). Sodium acetate concentrations were measured using high performance liquid chromatography (HPLC, Agilent 1200, Agilent Technology, USA) with an UV index detector and an Aminex HPX-87H column (Bio-Rad, USA). Separation during HPLC tests was achieved using a mobile phase of 1‰ (vol/vol) H_2SO_4 at a flow rate of 0.6 ml/min, a column temperature of 65 °C, and a detector temperature of 40 °C. Suspended solids (SS), volatile suspended solids (VSS) and total phosphorus (TP) were determined according to standard methods (APHA, 1995). The pH was measured using a WTW probe.

Total carbohydrate was measured after Maurer et al. (1997) by digesting activated sludge with a final HCl concentration of 0.6 M at 100 °C for 2 h, and the samples were mixed at intervals during digestion. After digestion and centrifugation, carbohydrate concentrations were measured by means of the sulfuric-phenol method (Dubois et al., 1956).

PHB concentration was detected by the HPLC according to Karr et al. (1983) using the modified procedure as follows: (i) 2 ml of mixed liquor was centrifuged at 14,000 rpm for 5 min; (ii) the centrifuged biomass was washed with 50%, 75% and 96% ethanol (each 3 min) to dehydrate the biomass; (iii) the biomass was then transferred to a screwed glass tube by washing with 0.5 ml concentrated sulfuric acid twice; (iv) the tube containing the dehydrated biomass and the 1 ml concentrated sulfuric acid was heated at 100 °C for 30 min; (v) sodium 3-hydroxybutyrate (Sigma–Aldrich, Ireland) was digested at the same condition as those samples and used for calibration; and (vi) the HPLC conditions used for PHB detection were the same as those used in the acetate detection. The PHB content was presented as the ratio of PHB/SS.

3. Results and discussion

3.1. System performance

In this study, EBPR was achieved after 11 days acclimation, with the effluent PO_4–P concentrations below 1 mg/l thereafter. The following batch experiments were carried out after more than 4 SRTs acclimation. With the influent PO_4–P concentration of 36.3 mg/l, the effluent PO_4–P concentration was below 0.9 mg/l, resulting in a percentage removal above 97.5%. The phosphorus content in the biomass was 12.8 ± 0.4%. Nitrification occurred in the reactor,