0273-2289/00/84–86/0981/$12.25

Optimal Production of Polyhydroxyalkanoates in Activated Sludge Biomass

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Abstract

Polyhydroxyalkanoates (PHAs) have been recognized as good candidates for biodegradable plastics, but their high price compared with conventional plastics has limited their use. In this study, activated sludge microorganisms from a conventional wastewater treatment process were induced, by controlling the carbon:nitrogen (C:N) ratio in the reactor liquor, to accumulate PHAs. In addition, an intermittent nitrogen feeding program was established to optimize the volumetric PHA productivity in a wastewater treatment process. The optimal overall polymer production yield of 0.111 g of polymer/g of carbonaceous substrate consumed was achieved under a C:N ratio of 96:1 by feeding nitrogen in the reactor liquor once every four cycles. At the same time, the amount of excess sludge generated from the wastewater treatment process was reduced by 22.9%.

Index Entries: Activated sludge; polyhydroxyalkanoates; carbon:nitrogen ratio; intermittent nitrogen feeding; wastewater treatment.

Introduction

In Hong Kong, an average of 8700 t of solid wastes from domestic, commercial, and industrial sources is disposed of at landfills each day *(1)* and approx 17% of the wet wt of these solid wastes is plastic materials, including packaging materials and disposable products. Commonly used packaging plastics are considered environmentally harmful because they are generally nonbiodegradable. Elimination of these nonbiodegradable

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plastics causes significant problems because the availability of landfills is limited, and incineration is bound to release greenhouse gases and toxic compounds. For these reasons, there has been considerable interest in developing biodegradable plastics that retain the desired physical and mechanical properties of conventional petrochemically derived plastics.

Among the various biodegradable plastics developed, polyhydroxyalkanoates (PHAs) have received much attention owing to their similar material properties to conventional plastics and complete biodegradability. In the early 1990s, the production and application of PHAs flourished and commercial products manufactured from PHAs have been developed. However, widespread application of PHAs is hampered by high production costs. Although much effort has been devoted to develop a process for economically producing PHAs, the price of Biopol, a copolyester of 3-hydroxybutyric acid and 3-hydroxyvaleric acid (P[3HB-co-3HV]), is still high: $4–8/kg compared to $0.6–0.9/kg for conventional synthetic plastic *(2,3)*.

For the production of PHAs on a commercial scale, the cost of microbial growth substrates is a critical factor. For the process with recombinant *Escherichia coli*, the cost of carbon source is 30.7% of the total cost *(4)*. From this viewpoint, several researchers have investigated the possibility of using industrial byproducts as the sole carbon source for economical PHA production. Liu et al. *(5)* successfully used beet molasses as the substrate to produce PHA by a recombinant *E. coli* HMS174/pTZ18u-PHB. Moreover, Yu et al. *(6)* demonstrated PHA production by *Alcaligenes latus* DSM 1124 using brewery malt waste as carbon substrate with specific polymer production yield of 0.7 g of polymer/g of cell. But the cost-effectiveness of such processes must be further investigated owing to the complexity of pretreating the raw materials.

In recent years, sludge microorganisms have been reported to accumulate PHA as an intermediate metabolic product from the uptake of organic matter in sewage *(7,8)*; therefore, it is possible to couple a wastewater treatment process with PHA production *(9)*. Furthermore, the quantity of excess sludge from a wastewater treatment process would be reduced as the PHA polymer in sludge is extracted for use, and, at the same time, the polymer production cost would be reduced by eliminating the substrate cost. A recent study used activated sludge microorganisms in a laboratory-scale wastewater treatment system. By controlling the carbon:nitrogen (C:N) ratio in the reactor liquor, PHAs were synthesized with a yield of 0.11 g of polymer/g of carbonaceous substrate consumed *(10)*. However, it was demonstrated that biomass growth was restricted under nitrogen-deficient conditions, which favors PHA accumulation. In prolonged nitrogen-deficient conditions in wastewater treatment, PHA production continued; however, cell growth and organic removal efficiency was adversely affected. Therefore, cell growth and PHA accumulation should be considered simultaneously to achieve a mutual benefit when designing wastewater treatment systems.

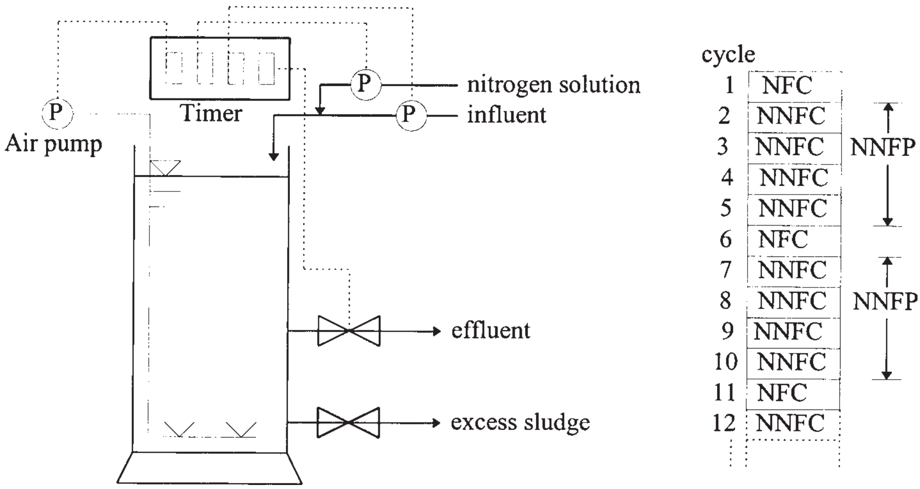

Fig. 1. Schematic diagram of the wastewater treatment process.

In this study, improving volumetric PHA productivity of activated sludge was investigated. For this purpose, an intermittent nitrogen feeding program was established to optimize the PHA production.

Materials and Methods

PHA Production by Activated Sludge Bacteria in Wastewater Treatment Process

Figure 1 shows a schematic diagram of the laboratory-scale sequential batch reactor (SBR) with a 12-L effective volume. The reactor was operated in a sequencing batch mode with a 2-h reaction time and a react-to-contact time ratio of 0.57. Each batch cycle contained several steps including influent feeding, 2-h aeration, settling, and effluent discharge. The batch loading rate was set at about 0.25 mg of chemical oxygen demand (COD)/(mg of MLVSS·d), and the average organic reduction efficiency was 98.3%. The reactor was seeded with activated sludge collected from a municipal sewage-treatment plant and was fed with a synthetic wastewater of an average COD of 2500 mg/L consisting of glucose. The wastewater was supplemented with NH_4Cl at 0.16 g/L to give a C:N ratio of 24:1. The wastewater was also supplemented with phosphorus, trace minerals, and a growth factor as described by Ho *(11)*.

When the reactor was operating under stable conditions, the supplied nitrogen concentration in the wastewater was reduced to give C:N ratios of 48:1, 96:1, and 144:1, creating different degrees of nutrient deficiency. Because prolonged nitrogen deficiency would adversely affect microbial growth and the organic treatment performance, for each C:N ratio above 24:1, an intermittent nitrogen feeding was required after every four cycles to enhance cell growth. In a nitrogen feeding cycle (NFC), the influent was supplemented with considerable amounts of nitrogen giving a C:N ratio of 24:1. The period between two NFCs was defined as a nitrogen nonfeeding period (NNFP), which consisted of multiple nitrogen nonfeeding cycles (NNFCs). The controlled sequence of feeding cycles is shown in Fig. 1.

Optimization of PHA Production

For the purpose of optimizing the volumetric PHA productivity, the biomass density in the reactor under different C:N ratios was increased by means of shortening the NNFP and increasing the NFC frequency. To develop a nitrogen feeding strategy for better polymer production during the wastewater treatment process, the reactor was operated with three other NNFPs (3:1, 2:1, and 1:1 NNFC:NFC) giving C:N ratios of 48:1, 96:1, and 144:1.

Sampling and Analytical Methods

The activated sludge samples of each set of cultures were periodically sampled from the reactor and analyzed for residual carbon concentration (COD) and dry cell mass, in accordance with standard methods *(12)*.

The polymers accumulated in the sludge biomass were extracted using a Soxhlet extractor with chloroform according to the procedure described by Lowell and Edwin *(13)* and modified by Chua et al. *(9)*. The weight of the extracted polymers was measured to determine the productivity. The extracted polymers were analyzed for composition according to the procedure described by Ho *(11)*.

Results and Discussion

The SBR system was operated under a C:N ratio of 24:1 for 120 d, and the steady-state performance reached 98% COD removal. Figure 2 shows the residual COD concentration and microbial cell mass in the reactor liquor during a 2-h reaction with a C:N ratio of 24:1. As the residual COD decreased, the cell mass in the system increased from 22.2 to 24.0 g. During the same period, the PHA content increased from 0.10 to 0.21 g (Fig. 3). This finding suggests that under nitrogen-limiting conditions, activated sludge microorganisms can make and accumulate PHA.

Effect of C:N Ratio on PHA Production

Figure 4 shows the effects of C:N ratio on specific growth yield ($Y_{X/S}$) and specific polymer yield ($Y_{P/X}$). As the C:N ratio increased from 24:1 to 144:1, the $Y_{X/S}$ decreased from 0.642 to 0.305 g of cell mass/g of COD consumed. However, increasing the C:N ratio also increased the $Y_{P/X}$ from 0.061 to 0.274 g of polymer/g of cell mass. This demonstrates that unfavorable cell growth conditions owing to nitrogen deficiency induce sludge microorganisms to accumulate intracellular carbon reserves in the form of PHA.

The specific polymer yield ($Y_{P/X}$), which reflects the PHA accumulation efficiency of microbial cells under different experimental conditions, is an important parameter in the microbial production of PHA. A larger $Y_{P/X}$

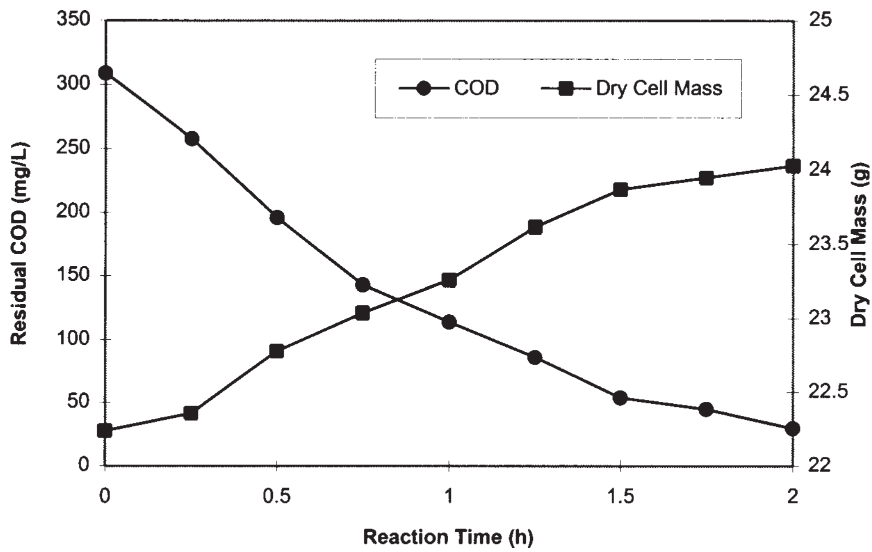

Fig. 2. Residual carbon and cell growth profiles under a C:N ratio of 24:1.

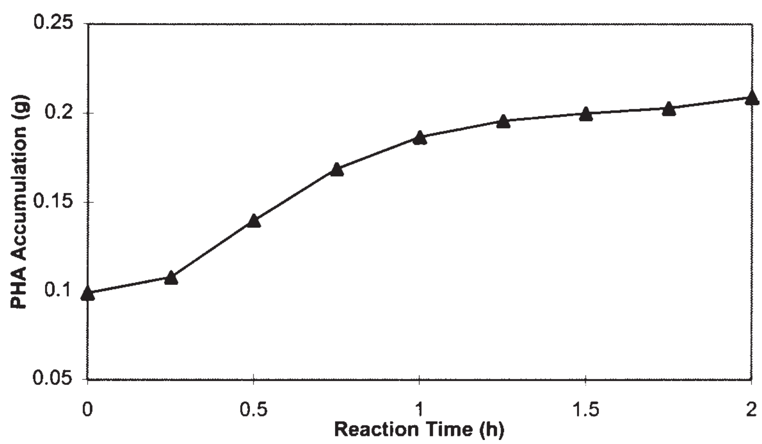

Fig. 3. Polymer accumulation under a C:N ratio of 24:1.

indicates that more PHA accumulates in the cell and less carbon source is used to generate cytoplasmic materials. However, for economical PHA production, a high volumetric productivity is required because it is an important factor that determines the production cost of PHA. The volumetric PHA productivity can be determined by the overall polymer production yield ($Y_{P/S}$), which is

$$Y_{P/S} = Y_{P/X} Y_{X/S} \tag{1}$$

Figure 4 shows that the overall polymer production yield ($Y_{P/S}$) reached a maximum of 0.093 g of polymer/g of COD consumed at a C:N ratio of 96:1 when the reactor was operated with 4:1 NNFC:NFC.

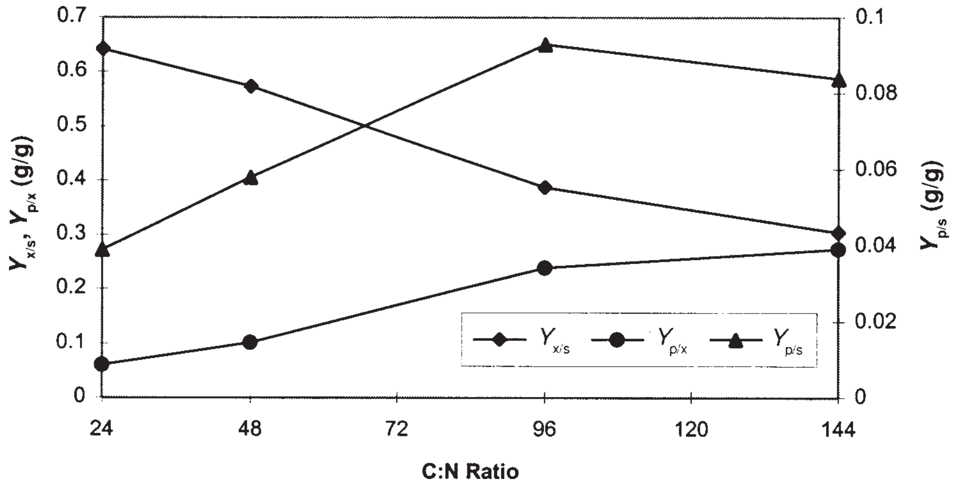

Fig. 4. Effect of C:N ratio on growth and polymer yields with 4:1 NNFC:NFC.

Table 1
Polymer Productivity Under Different C:N Ratios
and Different Intermittent Nitrogen Feeding Strategies

C:N ratio	NNFP (NNFC:NFC)	$Y_{X/S}$ (g/g)	$Y_{P/X}$ (g/g)	$Y_{P/S}$ (g/g)	COD removal efficiency (%)
24:1	—	0.642	0.061	0.039	98.8
48:1	4:1	0.573	0.102	0.058	98.4
48:1	3:1	0.604	0.094	0.057	98.7
48:1	2:1	0.604	0.077	0.047	98.7
48:1	1:1	0.588	0.064	0.038	98.1
96:1	4:1	0.387	0.240	0.093	97.8
96:1	3:1	0.485	0.229	0.111	97.8
96:1	2:1	0.531	0.132	0.070	97.7
96:1	1:1	0.575	0.087	0.050	98.3
144:1	4:1	0.305	0.274	0.084	96.2
144:1	3:1	0.351	0.256	0.090	96.0
144:1	2:1	0.439	0.204	0.089	98.0
144:1	1:1	0.474	0.069	0.033	98.1

Optimization of Polymer Production

To optimize further the volumetric PHA productivity, the biomass concentration was increased by shortening the NNFP. Table 1 shows the effect of shortening the NNFP on PHA productivity and organic reduction efficiency under different C:N ratios. For all three C:N ratios above 24:1, shortening the NNFP from 4:1 to 1:1 NNFC:NFC reduced the specific polymer yield ($Y_{P/X}$). As additional nitrogen was fed more frequently in the reactor, it reduced the nitrogen deficiency stress. Therefore, it is not surprising that activated sludge microorganisms accumulated less PHA with lower nitrogen deficiency.

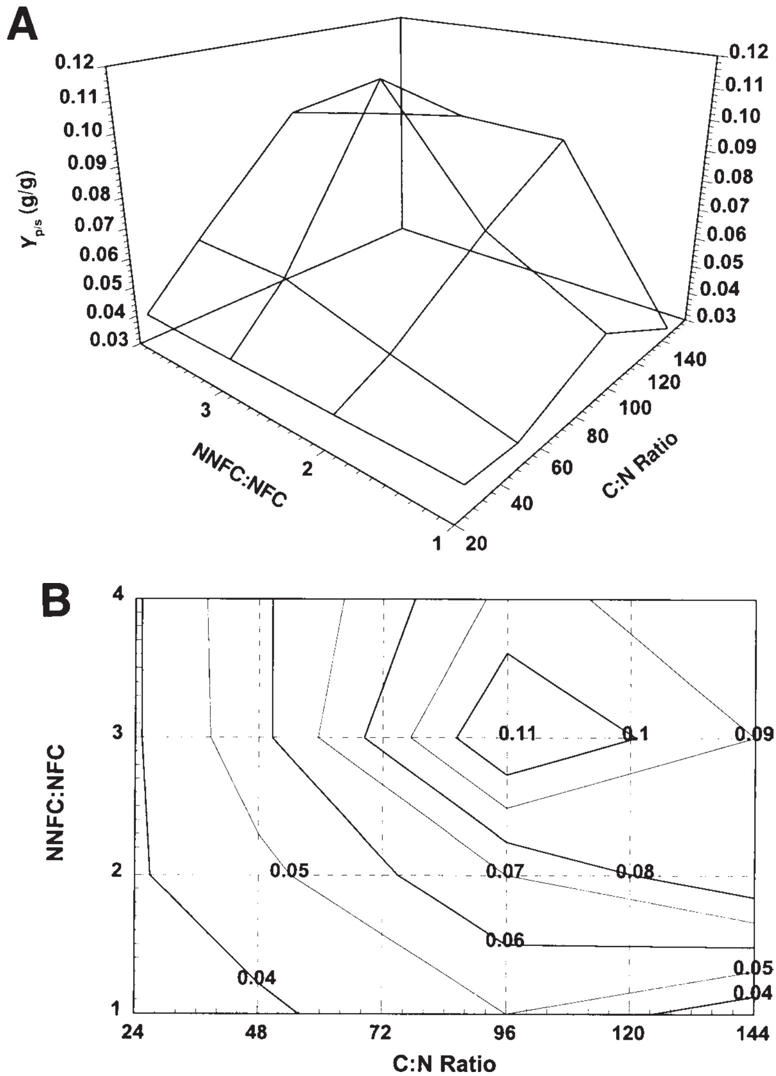

Fig. 5. **(A)** Overall polymer production yield with different intermittent nitrogen strategies. **(B)** Overview of (A). Data shown represent the $Y_{P/S}$ (g/g) of different contours.

When the C:N ratio was maintained at 48:1, shortening the NNFP from 4:1 to 1:1 NNFC:NFC, it decreased $Y_{P/X}$ from 0.102 to 0.064 g of polymer/g of cell mass, but did not significantly stimulate cell growth. As a result, the overall polymer production yield ($Y_{P/S}$) decreased from 0.058 to 0.038 g of polymer/g of COD consumed. When the C:N ratio was increased to 96:1, cell growth was significantly stimulated when additional nitrogen was supplied more frequently, which increased $Y_{X/S}$ from 0.387 to 0.575 g of cell mass/g of COD consumed. With 3:1 NNFC:NFC, $Y_{P/X}$ was 0.229 g of polymer/g of cell mass, which was not the highest. However, an optimal overall polymer production yield ($Y_{P/S}$) of 0.111 g of polymer/g of COD consumed was obtained under this operating condition (Fig. 5A,B). This phenomenon can be explained because higher cell densities steadily increase the total PHA concentration in the reactor, resulting in higher PHA productivity. However, when the NNFC:NFC ratio was further shortened from 2:1 or 1:1, $Y_{P/S}$ substantially decreased because microbial cells took up organic substrate for cell division rather than for PHA accumulation under the higher nitrogen concentration. Comparing the results of all three C:N ratios shows that a C:N ratio of 144:1 generally provided a higher $Y_{P/X}$ under different nitrogen feeding strategies. However, the residual biomass growth was very restricted compared with other C:N ratios; thus, a relatively high value of $Y_{P/S}$ (0.090 g of polymer/g of COD consumed) was obtained.

Because additional nitrogen was intermittently fed in the reactor liquor, microbial growth and organic reduction efficiency were not significantly affected by the SBR owing to different degrees of nitrogen deficiency. The COD removal efficiency remained above 96% for all cases investigated (Table 1).

Accumulated polymeric materials were extracted from the microorganisms and analyzed by gas chromatography. They were determined to contain mainly monomers of 3-hydroxybutyric acid and 3-hydroxyvaleric acid. The mole fractions of 3-hydroxybutyric acid in the polymer varied from 46 to 100% for different batches of product extracted.

Conclusion

Activated sludge microorganisms accumulated PHA by increasing the C:N ratio in the reactor liquor. The intermittent nitrogen feeding program optimized the volumetric PHA productivity. In addition, the requirements for treatment and disposal of the excess sludge produced from wastewater treatment could be reduced by extracting the polymer.

Acknowledgment

This work was funded by the Hong Kong Polytechnic University Research Committee under grant V 588.

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