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Coupling of Waste Water Treatment with Storage Polymer Production

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ABSTRACT

Storage polymers in bacterial cells can be extracted and used as biodegradable thermoplastics. However, widespread applications have been limited by high production costs. In this study, activated sludge bacteria in a conventional waste water treatment system were induced, by controlling the carbon–nitrogen (C:N) ratio in the reactor liquor, to accumulate storage polymers. Specific polymer yield increased to a maximum of 0.374 g polymer/g cell when the C:N ratio was increased from from 24 to 144, whereas specific growth yield decreased with increasing C:N ratio. An optimum C:N ratio of 96 provided the highest overall polymer production yield of 0.093 g of polymer/g of carbonaceous substrate consumed, without significantly affecting the organic treatment efficiency in the waste water treatment system.

Index Entries: Activated sludge; carbon–nitrogen ratio; poly-hydroxyalkanoates; storage polymer accumulation.

INTRODUCTION

In Hong Kong, 9500 t of municipal solid wastes are disposed of each day *(1,2)*. A very high proportion, 11 wt%, of these wastes are plastics packaging materials and disposable products. Plastics usage and plastics-waste generation are forecast to increase at 15% a year over the next decade *(3–5)*. These conventional plastics, which are synthetically derived from petroleum, are not easily decomposed in nature by microorganisms *(6,7)*, and are among the most environmentally harmful wastes *(1)*.

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In the past decades, there has been much interest in the development and production of biodegradable plastics as an environmentally friendly substitute for conventional plastics, particularly for packaging materials and disposable products. Various biodegradable plastics have been produced either by incorporating natural polymers into conventional plastics formulations, by chemical synthesis, or by microbial fermentation *(8)*. Among these biodegradable plastics, a family of more than 40 polyhydroxyalkanoates (PHAs) and their copolymeric derivatives have emerged as very attractive materials for their complete biodegradability *(9)*, wideranging physical properties by copolymerization, and biocompatibility to human tissue in surgical applications *(10,11)*. A number of bacteria, including *Alcaligenes* spp., *Pseudonomas* spp., recombinant *Escherichia coli*, and a number of filamentous genera accumulate these polymers or copolymers as an intracellular carbon reserve when unfavorable environmental conditions are encountered *(12)*. A nutrient-deficient condition could result in accumulated polymers of up to 75% of the dry cell mass *(13)*. These extracted and processed polymers have a number of properties that are comparable to commonly used plastics, namely thermoplastic processability and 100% water resistance. However, widespread application of PHAs is hampered by high costs of production.

Much effort has been spent in optimizing the PHA production process and reducing costs. Lee et al. investigated various recombinant *E. coli* using different complex culture media *(14)*. *E coli* strain XL1-blue in LB plus 20 g glucose/L could accumulate up to 0.369 g PHA/g glucose, or equivalent to 7 g PHA/L. Shirai et al. used a photosynthetic bacteria, *Rhodobacter spheroides*, in a fed-batch culture with glucose as the sole carbon to achieve a PHA production of 6 g/L *(15)*. Shimizu et al. used a cell-growth phase followed by a separately optimized nutrient-deficient PHA accumulation phase to improve the specific production yield to as high as 0.70 g PHA/g cell mass *(16)*. Despite these efforts, the current cost of PHA is still around 10 times higher than that of conventional plastics *(8)*.

This article reports a novel technique that induced the activated sludge bacteria in a conventional waste water treatment process to produce PHAs. This technique could significantly reduce the cost of PHA production and, at the same time, reduce the quantity of excess sludge that required further treatment.

METHODS

A reactor vessel with 10-L effective volume was seeded with returned activated sludge collected from municipal sewage treatment works. The reactor was fed with a synthetic waste water containing glucose at 4 g/L and NH_4Cl at 0.252 g/L, resulting in a C:N mass ratio of 24. The C:N ratio of 24 is widely accepted as required for normal bacterial synthesis in acti-

vated sludge processes *(17)*. The synthetic waste water was also supplemented with phosphorus, trace minerals, and a growth factor with the following formulation in g/L: KH_2PO_4, 0.0037; $MgSO_4 \cdot 7H_2O$, 0.0200; $FeCl_3$, 0.0284; $MnCl_2 \cdot 2H_2O$, 0.0003; $Al_2(SO_4)_3 \cdot 18H_2O$, 0.0022; $CaCl_2$ 0.0400; $CoCl_2 \cdot 6H_2O$, 0.0080; $NaSiO_3 \cdot 5H_2O$, 0.0040; H_3BO_3, 0.0040; $ZnSO_4 \cdot 7H_2O$, 0.0020; $CuSO_4 \cdot 5H_2O$, 0.0020; (NH_4) $2MoO_4$, 0.0020; thiamine hydrogen chloride, 0.0080.

The reactor was operated in a sequencing batch mode with a batch loading rate of 0.4 mg COD/mg MLVSS-d. The react-to-contact time ratio was 0.6, and the average organic reduction efficiency was 98.1%. The detailed operation and performance of the sequencing batch reactor (SBR) system were described by Ho *(18)*. When the reactor was operating under stable conditions, the nitrogen concentration in the synthetic waste water was sporadically reduced to result in C:N mass ratios of 48, 96, and 144, creating different degrees of nutrient deficiency.

When stable operation was attained in the SBR under each C:N ratio, samples were periodically collected and analyzed during the 2-h reaction time in one randomly selected SBR operation cycle. The samples were analyzed for total organic carbon (TOC), total kjeldahl nitrogen (TKN), dissolved oxygen, pH, and dry cell mass. The analytical techniques were carried out according to the standard methods *(19)*. The mass of PHA extracted by 1,1,2-trifluoro-1,2,2-trichloroethane from the cell mass was also measured. The organic solvent extraction and precipitation procedure for PHA was in accordance with that described by Suzuki et al. *(20)*.

RESULTS AND DISCUSSION

The SBR was in operation for 180 d and was consistently treating the synthetic waste water with an organic reduction efficiency around 98.1%. Residual organic and nutrient concentrations, cell growth, and polymer accumulation, with a C:N ratio of 24, are summarized in Table 1. The profiles of depletion of carbon, measured as TOC, and nitrogen, measured as TKN, in the reactor liquor are shown in Fig. 1. Both the TOC and TKN maintained a consistent depletion rate throughout the 2-h reaction time. The dissolved oxygen profile corresponded with the microbial activities in the activated sludge during the reaction. The pH of the reactor liquor remained between 6.50 and 6.91. There was a net cell growth of 1.74 g in the reactor, as measured in dry cell mass, and an accumulation of 0.11 g of intracellular storage polymer (Fig. 2).

On the other hand, when the C:N ratio was increased to 96, the TKN was almost depleted within the first 15 min, and the reactor entered into nitrogen-deficient condition (Fig. 3). The characteristics in the reactor are summarized in Table 2. The net cell growth of 0.92 g in the reactor was lower than that when the C:N ratio was 24. However, the accumulation of

Table 1
Reactor Operated Under C:N Ratio of 24

React time, h	Residual TOC, mg/L	Residual TKN, mg/L	D.O., mg/L	pH	Dry cell mass, g	Polymer accumulation, g
0	274	12.6	3.23	6.91	42.85	0.01
0.25	228	8.3	4.81	6.64		
0.50	179	5.9	4.52	6.61	43.58	0.04
0.75	135	5.3	1.33	6.59		
1.00	124	4.7	0.27	6.56	43.79	0.09
1.25	72	4.0	0.08	6.50		
1.50	44	3.0	0.35	6.56	44.59	0.12
1.75	31	2.8	0.42	6.58		

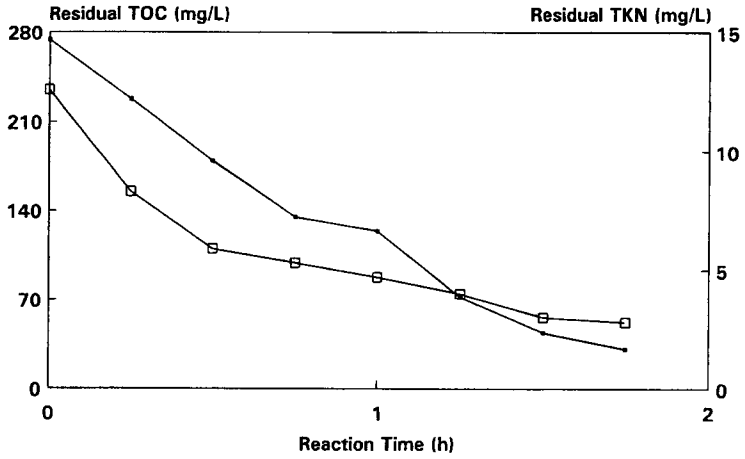


Fig. 1. Carbon and nitrogen profiles under C:N ratio of 24. -■- TOC, -⊟- TKN.

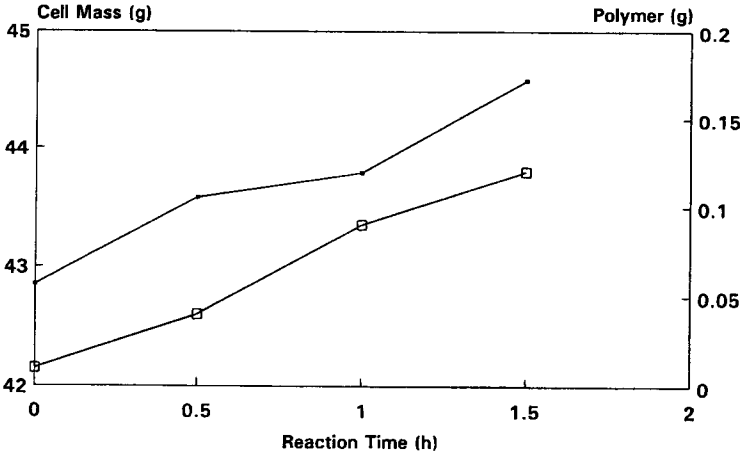


Fig. 2. Growth and polymer accumulation under C:N ratio of 24. -■- Cell mass, -⊟- polymer.

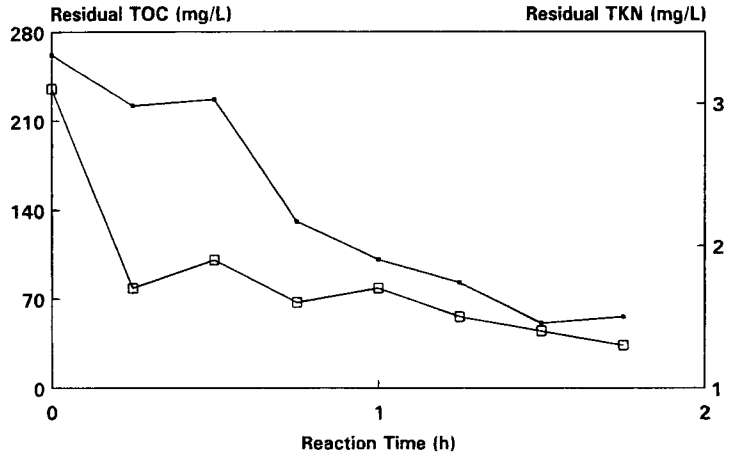


Fig. 3. Carbon and nitrogen profiles under C:N ratio of 96. -■- TOC, -⊟- TKN.

Table 2
Reactor Operated Under C:N Ratio of 96

React time, h	Residual TOC, mg/L	Residual TKN, mg/L	D.O., mg/L	pH	Dry cell mass, g	Polymer accumulation, g
0	262	3.1	3.46	6.92	40.68	0.17
0.25	222	1.7	3.06	6.91		
0.50	227	1.9	2.32	6.88	40.68	0.15
0.75	131	1.6	1.67	6.89		
1.00	101	1.7	0.07	6.73	40.91	0.23
1.25	83	1.5	1.25	6.84		
1.50	51	1.4	1.32	6.87	41.60	0.40
1.75	56	1.3	1.18	6.90		

0.23 g of intracellular storage polymer was more than that when the C:N ratio was 24. The rate of polymer accumulation increased significantly after 0.5 h of reaction time (Fig. 4), which was 15 min after the reaction entered into nitrogen deficiency.

Overall organic consumption, cell growth, polymer accumulation, and yields during the 2-h reaction under four different C:N ratios are summarized in Table 3. An increase in C:N ratio from 24–144 resulted in a decline in specific growth yield, $Y_{x/s}$, from 0.579–0.232 g cell mass/g TOC (Fig. 5). This indicated that a nitrogen-deficient condition affected the growth of biomass in the activated sludge. On the other hand, the increased C:N ratio caused an increased specific polymer yield or intracellular polymer fraction, $Y_{p/x}$, from 0.066–0.374 g polymer/g cell mass. This demonstrated that the unfavorable condition resulting from nitrogen deficiency induced the microorganisms in the activated sludge to accumulate

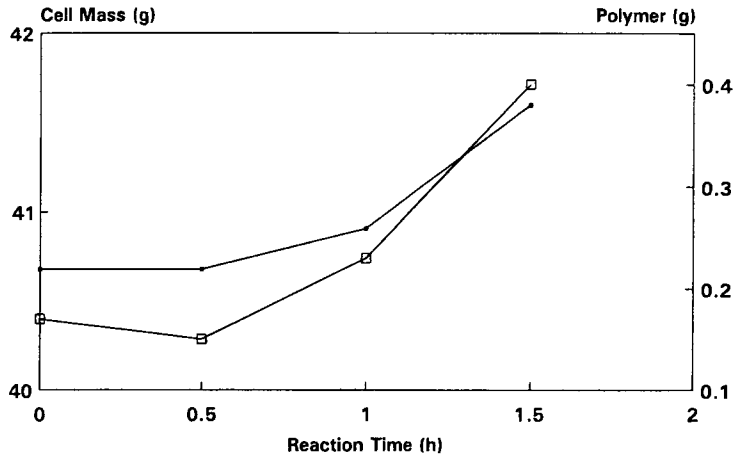


Fig. 4. Growth and polymer accumulation under C:N ratio of 96. -■- Cell mass, -⊟- polymer.

Table 3
Polymer Productivity Under Different C:N Ratios[a]

C:N ratio	*del* X, g	*del* P, g	*del* S, g	$Y_{X/S}$, g/g	$Y_{P/X}$, g/g	$Y_{P/S}$ g/g	TOC removal efficiency, %
24	1.74	0.11	2.92	0.597	0.066	0.038	98.1
48	1.51	0.18	3.11	0.485	0.122	0.058	98.1
96	0.92	0.23	2.47	0.374	0.246	0.093	96.5
144	0.57	0.21	2.47	0.232	0.374	0.085	96.9

del X = net cell growth as measured in dry cell mass during the 2-h reaction time;
del P = net accumulation of intracellular polymers during the 2-h reaction time;
del S = net consumption of TOC during the 2-h reaction time.

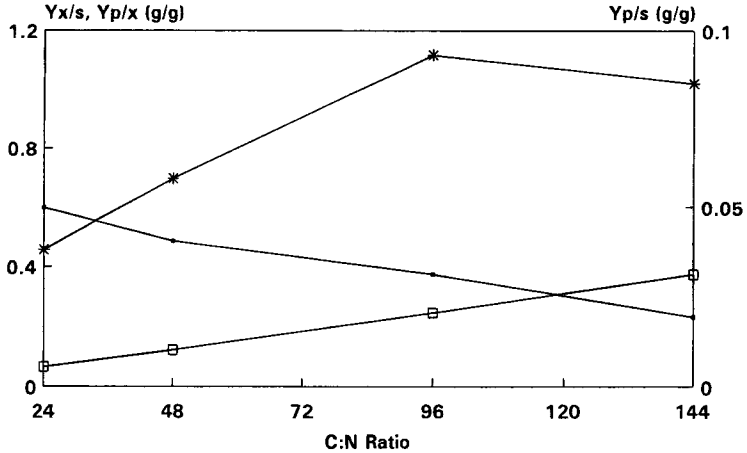


Fig. 5. Growth and polymer yields under different C:N ratios. -■- $Y_{x/y}$ -⊟- $Y_{p/x}$, -✳- $Y_{P/S}$.

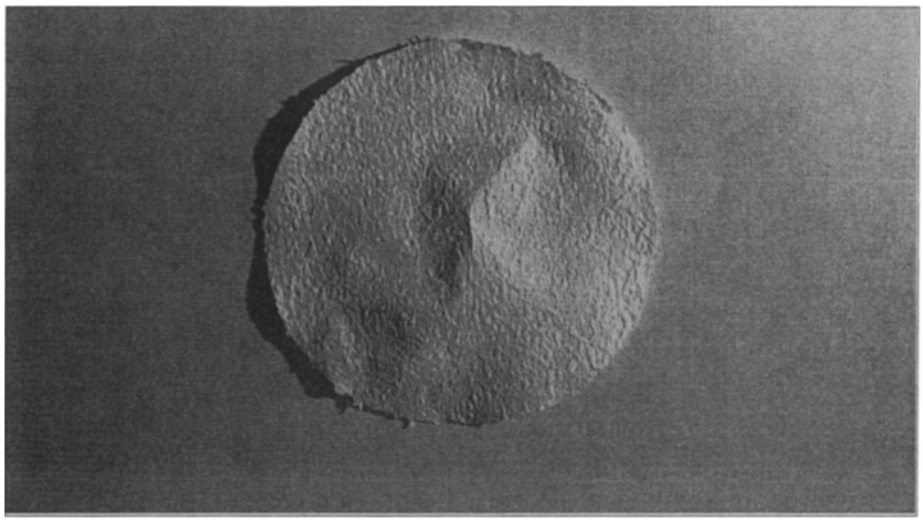
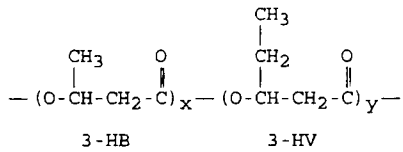


Fig. 6. Polymeric materials extracted from activated sludge.



where x:y ratio varied from 0.79 to 5.67

Fig. 7. Structural formula of copolymers.

more intracellular storage polymers. The overall polymer production yield, $Y_{p/s}$, which was a product of $Y_{x/s}$ and $Y_{p/x}$, reached a maximum of 0.093 g polymer/g TOC under the C:N ratio of 96.

Accumulated polymeric materials extracted from the microorganisms and precipitated are shown in Fig. 6. These polymeric materials were analyzed by gas chromatographic methods to contain mainly poly-β-hydroxybutyric acid, and copolymers of β-hydroxybutyric (3-HB) and β-hydroxyvaleric (3-HV) acids *(18)*. The structural formula of these copolymers is shown in Fig. 7. The intracellular polymer fraction, $Y_{p/x}$, was 0.374 g polymer/g cell mass, indicating that 37.4 wt% of the activated sludge was composed of the polymers. If this portion was extracted for use, the requirements for treatment and disposal of the excess sludge produced from the waste water treatment process would be reduced by 37.4%.

Sporadic changes of C:N ratio in the synthetic waste water above 24, creating a nitrogen-deficient condition in the activated sludge, did not significantly affect the efficiency of organic reduction by the SBR. The TOC removal efficiency remained above 96.5% for all the C:N ratio investigated

(Table 3). These observations were in contrast with the widely accepted view that C:N ratio in activated sludge processes must be kept around 24 in order to enable normal microbial cell synthesis *(17)*. Nitrogen deficiency would result in a slowdown in microbial growth, which would, in turn, have an adverse effect on the organic treatment performance by the process. However, it must be noted that if the nitrogen-deficient condition in the SBR was prolonged to have a continual polymer production, cell growth and TOC removal efficiency would have been adversely affected. Therefore, an intermittent nitrogen feeding program must be established in order to optimize the polymer production without significantly affecting the normal treatment performance of the activated sludge process.

CONCLUSION

Activated sludge bacteria were induced by controlling the C:N ratio in the SBR reactor liquor to accumulate storage polymers. Specific polymer yield increased with increasing C:N ratio, whereas specific growth yield decreased with increasing C:N ratio. An optimum C:N ratio of 96 provided the highest overall polymer production yield. Sporadic adjustments of the C:N ratio did not significantly affect the treatment efficiency in the SBR. Production and recovery of PHAs from activated sludge could significantly reduce the cost of PHAs and, at the same time, reduce the quantity of excess sludge that required further treatment.

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