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Production of Biological Polymers from Organic Wastes

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SUMMARY: Biologically-produced polymers, from microbial fermentation are naturally biodegradable and are potential environment-friendly substitutes for some synthetic plastics. However, broader applications are restricted by the high production costs and limitations in physical and mechanical properties. In this study, activated sludge bacteria in a conventional wastewater treatment system treating a wastewater that contained organic pollutants, were induced by nitrogen deficiency to accumulate intracellular storage polymers, which can be extracted as a low-cost source of biodegradable plastics. Chromatographic analysis of the extracted polymers revealed a composition of poly-hydroxyalkanoate and a number of related co-polymers. *Alcaligene spp.* in the activated sludge microbial consortium was identified as the main genus accumulated these polymers. When the C:N ratio was increased from 20 to 140, the specific polymer yield increased to a maximum of 0.39 g polymer/g dry cell while specific growth yield decreased to 0.26 g dry cell/g carbonaceous matter consumed. The highest overall polymer production yield of 0.11 g polymer/g carbonaceous matter consumed was achieved when the C:N ratio was maintained at a nitrogen-deficient level of 100. The specific polymer yield in the isolated *Alcaligene spp.* cells were as high as 0.7 g polymer/g dry cell mass. The composition of the co-polymers, and hence the physical and mechanical properties, could be controlled by manipulating the influent organic compositions.

Introduction

In Hong Kong, plastic packaging materials and disposable products account for over 15 weight percent of the 10,000 tonnes of municipal solid wastes produced each day[1,2)]. These conventional synthetic plastic materials that are derived from the petro-chemical industry are not naturally biodegradable and are environment-harmful wastes[3,4)]. While plastic-waste generation is forecast to increase at 15 % per year over the next decades[5-7)], there has been much interest in developing environment-friendly materials as plastic substitutes[8,9)]. Amongst the variety of biodegradable plastics, a family of more than 40 poly-hydroxyalkanoates (PHAs) and their co-polymeric derivatives (Fig. 1) has emerged since the 1980s as very

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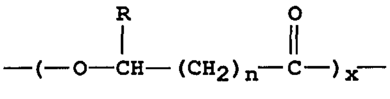
attractive materials due to their complete biodegradability[10,11]), appropriate physical and mechanical properties after co-polymerization, and biocompatibility to human tissue in surgical applications[12,13]). A number of bacteria, including *Alcaligenes spp.*, *Pseudomonas spp.*, recombinant *Escherichia coli* and a number of filamentous genera, accumulate these polymers or co-polymers as an intracellular carbon reserve when unfavourable nutrient-deficient conditions are encountered[14]). 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, broader application of PHAs is hampered by high production costs.



$$-(-O-\overset{\overset{R}{|}}{CH}-(CH_2)_n-\overset{\overset{O}{\|}}{C}-)_x-$$

R = n-alkyl pendant group of variable chain length

PHA Family Members	**R Group**	**n**
3-HB, 3-hydroxybutyrate	*methyl group*	*1*
3-HV, 3-hydroxyvalerate	*ethyl group*	*1*
3-HC, 3-hydroxycaproate	*n-propyl group*	*1*
3-HH, 3-hydroxyheptanoate	*n-butyl group*	*1*
3-HO, 3-hydroxyoctanoate	*n-pentyl group*	*1*
3-HN, 3-hydroxynonanoate	*n-hexyl group*	*1*
3-HD, 3-hydroxydecanoate	*n-heptyl group*	*1*
3-HUD, 3-hydroxyundecanoate	*n-octyl group*	*1*
3-HDD, 3-hydroxydodecanoate	*n-nonyl group*	*1*
4-HV, 4-hydroxyvalerate	*methyl group*	*2*
4-HC, 4-hydroxycaproate	*ethyl group*	*2*

Fig. 1 Formula of poly-hydroxyalkanoates and co-polymeric derivatives

In the past decade, much effort has been spent in optimizing the PHA production process and using less costly raw materials[15-17]). Despite these efforts, the current cost of PHA is still around six times that of conventional plastics[8]). Substantial cost reduction is necessary before widespread applications in packaging and disposable products are feasible[18,19]). In previous studies by Chua *et al.*[10,20]), activated sludge bacteria in wastewater treatment systems were shown to be accumulating PHA-related storage polymers under specific range of carbon-nitrogen (C:N) ratio in the reactor liquor. This could represent an important step towards PHA cost reduction.

This paper describes a novel technique that induced the activated sludge bacteria in a wastewater treatment process to convert xenobiotic organic constituents in a chemical industrial wastewater to PHAs. This technique could significantly reduce the costs of PHA production and, at the same time, produced a wide range of different PHAs with different co-polymeric composition.

Methods

Activated sludge from a municipal sewage treatment works was cultured in a 20-L sequencing batch reactor (SBR) to treat a chemical process wastewater. The wastewater was of a typical Chemical Oxygen Demand (COD) content of 2,500 mg/L, comprising of carboxylic acids, namely butyric and valeric acids. The wastewater was supplemented with ammonium chloride at 0.48 g/L to result in a C:N mass ratio of 20, which is required for normal bacterial synthesis in activated sludge. The reactor was operated in a sequencing batch mode with a 2-hour reaction time and a react-to-contact time ratio of 0.57. The batch loading rate was set at around 0.25 mgCOD/mgMLVSS-d (+/- 13 %) and the average organic reduction efficiency was 90 %. The detailed operation cycle and performance of the SBR system have been described by Ho21). When the reactor was operating under stable conditions, the nitrogen concentration in the wastewater was progressively reduced to result in C:N mass ratios of 40, 60, 80, 100, 120 and 140, creating different degrees of nutrient deficiency.

When stable SBR operation was attained under each C:N ratio, samples were periodically collected during the 2-hour reaction time in a randomly selected operation cycle. The samples were analysed for COD, Total Kjeldahl Nitrogen (TKN), Dissolved Oxygen (DO), pH and Dry Cell Mass (DCM). The analyses were carried out according to the Standard Methods22). The mass of Intracellular Storage Polymers (ISPs) extracted by Freon (1,1,2-trifluoro-1,2,2-trichloroethane) from the cell mass was also measured. The organic solvent extraction and precipitation procedure for the ISPs was in accordance to that described by Suzuki *et al.*23) and modified by Yu *et al.*24). The composition of the ISPs extracted was analysed by gas chromatographic method and the microbial genus that accumulated the polymers was observed by microscopic method, both of which have been described by Yu *et al.*24).

Results and Discussion

Cell Growth and Polymer Accumulation

The SBR was operated and maintained with an organic reduction efficiency of around 90 % for 3 months. Residual organic carbon (COD) and nitrogen (TKN) concentrations, cell growth (DCM) and polymer accumulation (ISP) in the 2-hour reaction time of the SBR operating cycle under various C:N ratios are shown in Fig. 2 and 3. Both the COD and TKN maintained a consistent depletion rate under a C:N ratio of 20. The DO profile corresponded to the microbial activities during the reaction. The pH of the reactor liquor was not controlled and fluctuated between 6.50 and 7.00. There was a net cell growth of 1.80 gDCM in the reactor, and an accumulation of 0.10 gISP. When the C:N ratio was increased to 40, 60 and 80 similar observations were made on the trends of residual COD, TKN, DO and pH. However, the net cell growth decreased to 1.50 gDCM and the storage polymer accumulation increased substantially to 0.20 gISP.

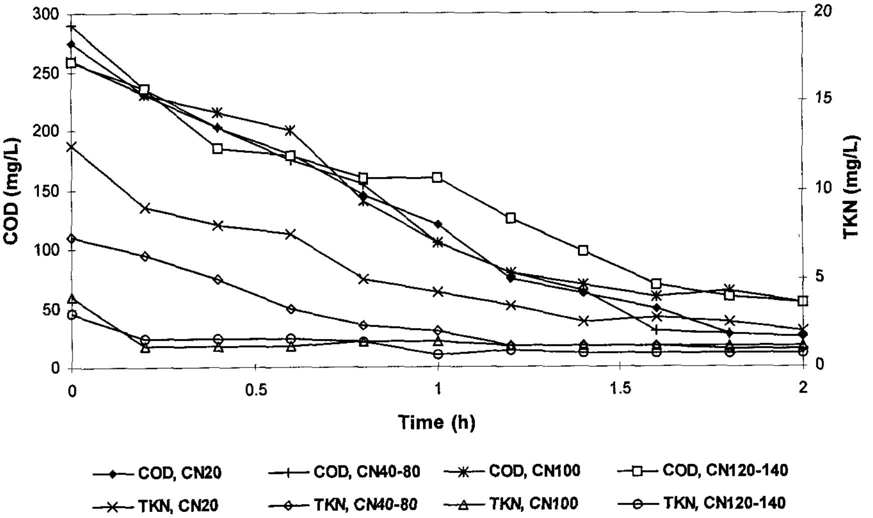


Fig. 2 Carbon and nitrogen consumption under various C:N ratios

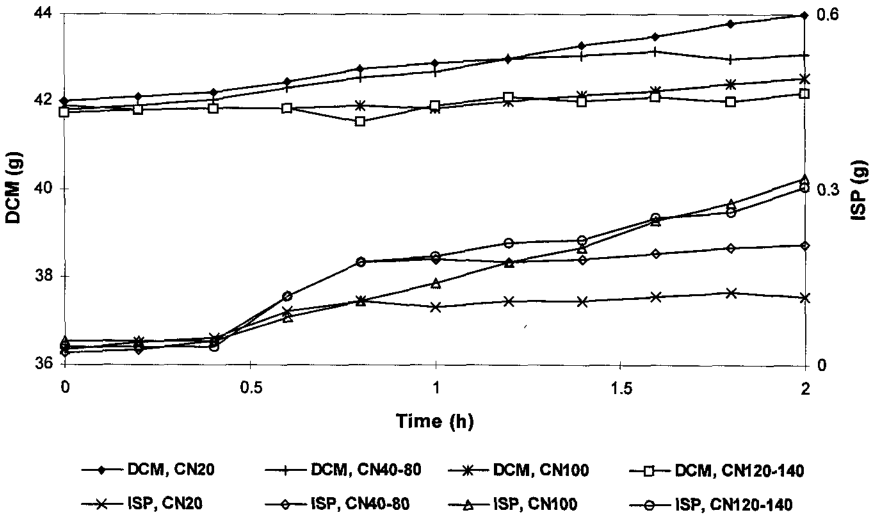


Fig. 3 Cell growth and polymer accumulation under various C:N ratios

When the C:N ratio was increased to 100, the TKN was almost depleted within the first 30 minutes, and the reactor entered into nitrogen-deficient condition throughout the remaining reaction time. The net cell growth of 0.9 gDCM was substantially lower than those with lower C:N ratios. However, the accumulation of 0.25 gISP of intracellular storage polymer was more than that when the C:N ratios were below 80. The rate of polymer accumulation increased significantly after 30 minutes of reaction time, which was when the reaction entered into nitrogen deficiency. When the C:N ratio was further increased to 120 and 140, the net cell growth decreased to between 0.6 and 0.7 gDCM and the polymer accumulation remained almost unchanged.

The overall organic consumption (del S), cell growth (del X), polymer accumulation (del P), and growth and product yields during the 2-hour reaction under various C:N ratios are shown in Fig. 4. An increase in C:N ratio from 20 to 140 resulted in a decline in specific growth yield, SGY, from 0.75 to 0.26 gDCM/gCOD. This indicated that the nitrogen-deficient condition affected the normal growth and cytoplasmic synthesis 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, SPY, from 0.05 to 0.39 gISP/gDCM. This demonstrates that the unfavourable nitrogen-deficient condition induces the microorganisms in the activated sludge to accumulate more intracellular carbon reserve in the form of storage polymers. The overall polymer production yield PPY, which is a product of SGY and SPY, reached a maximum of 0.11 gISP/gCOD under the C:N ratio of 100. These results are comparable to those found in earlier studies in our laboratory using a similar technique in the SBR system to treat a synthetic wastewater containing 4 g/L of glucose, brewery waste, soy fermentation waste and other food-industrial wastes10,20).

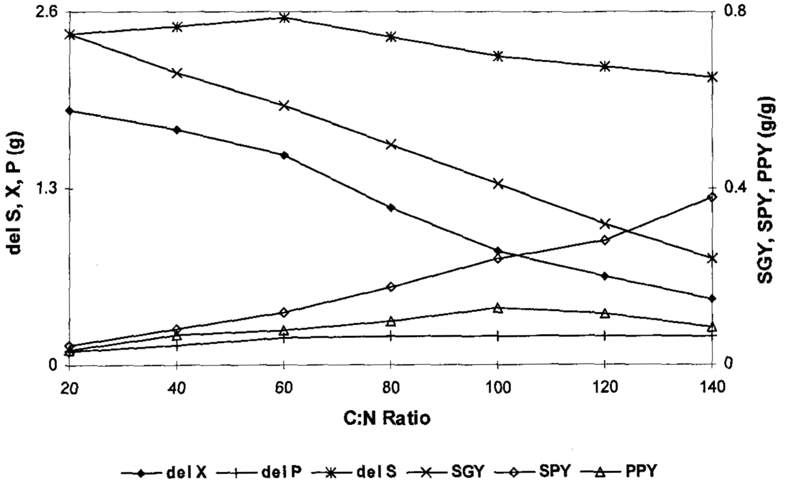


Fig. 4 Polymer productivity under various C:N ratios

The intracellular polymer fraction, SPY, could reach a highest value of 0.39 gISP/gDCM, indicating that 39 weight % of the activated sludge biomass was composed of the polymers. If this portion was extracted for use as biodegradable plastics, the requirements for treatment and disposal of the excess sludge produced from the wastewater treatment process could be reduced by a similar percentage.

The nitrogen-deficient conditions in the activated sludge when the C:N ratio in the chemical wastewater was above 20 did not significantly affect the efficiency of organic reduction by the SBR. The COD removal efficiency remained above 90 % for all the C:N ratios investigated. These observations were in contrast with the widely accepted view that the C:N ratio in activated sludge processes must be kept around 20 in order to enable normal microbial cell synthesis. This observation was attributed to the fact the nitrogen deficient condition was not introduced in a prolonged manner. The sporadic nitrogen deficiency did not result in a slow down in microbial growth, which in turn, did not have any adverse effect on the organic treatment performance by the process. However, it must be stressed that if the nitrogen-deficient condition in the SBR was prolonged to have a continual polymer production, cell growth and COD removal efficiency would have been adversely affected. Therefore, an intermittent nitrogen feeding programme must be established in order to optimise the polymer production without significantly affecting the normal treatment performance of the activated sludge process.

The main bacterial genus in the activated sludge that accumulated the polymers was *Alcaligenes spp.* (Fig. 5). While specific polymer yield, SPY, in activated sludge was 0.39 gISP/gDCM, that in *Alcaligenes spp.* could reach as high as 0.70 gISP/gDCM. This value is comparable with that found by Yu *et al.*[24)] and Shimizu *et al.*[16)] using pure or mono-culture fermentation processes.

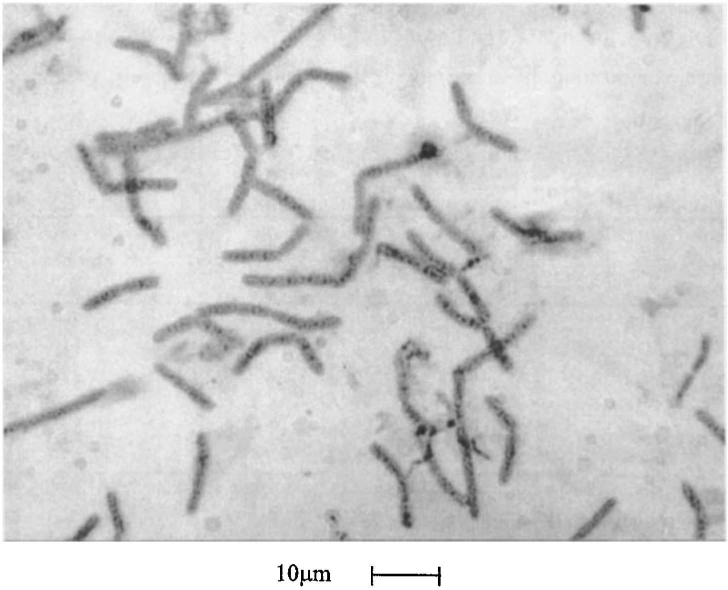


Fig. 5 *Alcaligenes spp.* that accumulated polymers

Effects of Influent Organic Composition on Co-Polymer Composition

When butyric acid was used as the sole carbon source, there was only PHB (poly-hydroxybutyrate) homo-polymer produced instead of PHBV (poly-hydroxybutyrate-co-valerate) co-polymer (Table 1). On the other hand, the highest 3HV mole fraction in the co-polymer accumulated was when valeric acid was used as sole carbon source. The mole fraction of 3HV in the accumulated co-polymer increased proportionately with the valeric acid concentration in the medium. This linear relationship between 3HV mole fraction in co-polymer and the valeric acid concentration may be correlated through a linear expression. If the 3HV-mole fraction was represented by Y1 and the valeric acid concentration in the influent was represented by X1, then Equation (1) applies.

$$\mathbf{Y1 = 0.464\ X1 + 0.400 \qquad Sq.r = 0.956 \qquad (1)}$$

These results from activated sludge were in agreement with that observed in pure cultures of *A. eutrophus*[25,26)]. These results indicated that the 3HV mol fraction of the PHBV co-polymer accumulated in activated sludge could be controlled by adjusting the valeric acid concentration in the medium. The linear fit of the Y1-X1 correlation also indicated that the co-polymeric composition in actual production could be accurately predicted from the medium composition.

Table 1 Copolyester Accumulation under Different Carboxylic Acid Ratios

Carbon Conc. (g/L)		C_4 to C_5 Ratio	Yp/x[1] (g/g)	Yp/s[2] (g/g)	HV Fraction (mol %)	Tm (°C)
C_4	C_5					
3.0	0.0	100:0	0.41	0.41	0	177.6
2.4	0.6	80:20	0.36	0.18	11	149.0
1.8	1.2	60:40	0.32	0.15	27	136.0
1.2	1.8	40:60	0.26	0.12	33	129.5
0.6	2.4	20:80	0.06	0.10	41	116.0
0.0	3.0	0:100	0.04	0.06	43	99.0

[1] Specific copolyester yield was calculated as the mass of copolyesters accumulated during the nutrient-deficient stage divided by the cell mass.

[2] Copolyester production yield was calculated as the mass of copolyesters accumulated during the nutrient-deficient stage divided by the TOC consumed.

Thermal Properties of the Co-Polymer

The melting temperature, *Tm*, of the co-polymer accumulated in activated sludge with different medium composition ranged from 99 to 177.6 °C (Table 1). The co-polymers with maximum and minimum values of *Tm* were obtained when butyric acid and valeric acid were respectively used as the sole carbon source in the medium. Increase of 3HV monomeric units in the PHBV co-polymer formulation resulted in a close to proportionate decrease of polymer melting temperature. If the 3HV mole fraction in the co-polymer was represented by X2, then Equation (2) applies.

$$\mathbf{Tm = 1.547\ X2 + 174.470 \qquad Sq.r = 0.938 \qquad (2)}$$

These results agree with the published thermal properties of polymers and co-polymers of PHA. Intracellular polymers of PHA are highly crystalline thermoplastics with a melting temperature (*Tm*) of around 180 °C for PHB homo-polymer and a range from 96 to 160 °C for PHBV co-polymers depending on the fraction of 3HV units27). Therefore, the 3HV mol fraction of the PHBV co-polymer accumulated in activated sludge, and hence its thermal and mechanical properties, namely tensile, compressive, flexural and shear strengths, could be controlled by adjusting the valeric acid concentration in the medium. The measurements and detailed data on co-polymeric compositions and physical properties have been described by Chua *et al.*28). Samples of the accumulated polymeric materials extracted from the activated sludge microorganisms and subsequently precipitated is shown in Fig. 6.

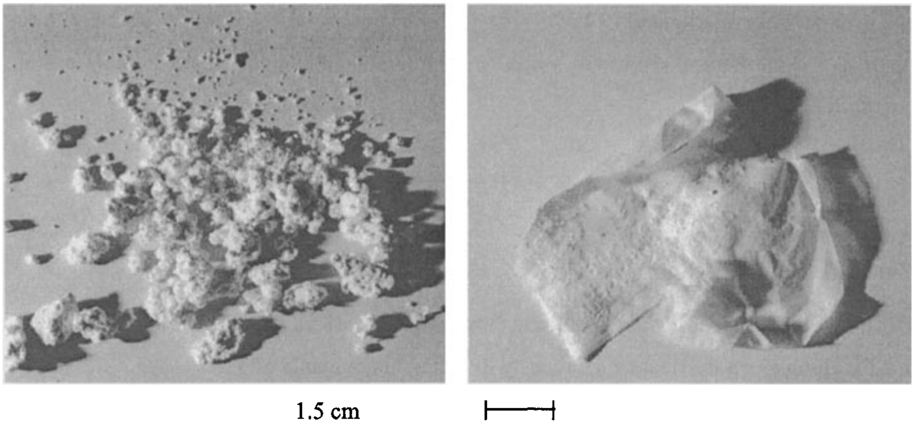
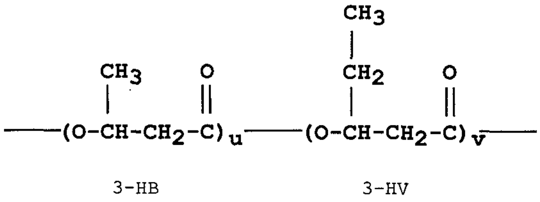


Fig. 6 Polymers extracted from activated sludge
(left, HV mol%=0; right, HV mol%=43)



$$-\left(O-\overset{CH_3}{\overset{|}{CH}}-CH_2-\overset{O}{\overset{\|}{C}}\right)_u-\left(O-\overset{CH_3}{\overset{|}{\overset{CH_2}{\overset{|}{CH}}}}-CH_2-\overset{O}{\overset{\|}{C}}\right)_v-$$

where u/(u + v) varied from 0.5 to 1.0

Fig. 7 Structural formula of extracted polymers

Conclusion

Activated sludge microorganisms in a chemical wastewater treatment system accumulated storage polymers under nitrogen-deficient conditions. Specific polymer yield increased with increasing C:N ratio, while specific growth yield decreased with increasing C:N ratio. An optimum C:N ratio of 100 provided the highest overall polymer production yield. Sporadic adjustments of the C:N ratio did not significantly affect the treatment efficiency. This novel technique to produce and recover PHAs from activated sludge provided a potentially inexpensive source of biodegradable plastics and, at the same time, reduced the quantity of excess sludge that required further treatment. The main bacterial genus that accumulated the polymers was identified as *Alcaligenes spp.*. The accumulated polymeric materials were mainly poly-beta-hydroxybutyric acid, and co-polymers of beta-hydroxybutyric acid (3-HB) and beta-hydroxyvaleric acid (3-HV). The composition of the co-polymers, and hence the physical and mechanical properties, could be controlled by manipulating the influent organic compositions.

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