第42卷 增 刊 中山大学学报(自然科学版) Vol.42 Suppl.
2003年 6月 ACTA SCIENTIARUM NATURALIUM UNIVERSITATIS SUNYATSENI Jun. 2003

Polyhydroxyalkanoates of Different Structure Synthesized by Activated Sludge with Acetate and Propionate as Sole Carbon Source*

LIN Dong-en[1], ZHANG Yi-wei[1], SUN Dong-cheng[1], SHEN Jia-rui[2]
(1. *Department of Applied Chemistry*, *South China University of Technology*, *Guangzhou* 510641, *China*;
2. *College of Material Science and Engineering*, *South China University of Technology*, *Guangzhou* 510641, *China*)

Abstract: Polyhydroxyalkanoates(PHAs) are Polyesters synthesized by numerous bacteria in sludge as intracellular carbon and energy storage compounds under the condition of unbalance growth. Another important aspect of PHAs is thermoplastic polyester with the remarkable characteristics of biodegradability. In this paper, in order to research the relations of carbon sources to structure of PHAs, using acetate(Ⅰ) and propionate(Ⅱ) as the unique carbon source, two kinds of PHAs samples were obtained by assimilated sludge as PHAs synthesis strains in the anaerobic process. Their structures were determined by ^{1}H NMR ^{13}C NMR and GC-MS. When the carbon source was acetate, the unit composition of PHAs was 93.91% (mole fraction, x) 3-hydroxybutyrate (3HB) and $x = 6.09\%$ 3-hydroxyvalerate(3HV); When the carbon source was propionate, the unit composition of PHAs was $x = 28.66\%$ 3HB, $x = 63.13\%$ 3HV, $x = 2.55\%$ 2-methy-3-hydroxybutyrate(2M3HB) and $x = 5.66\%$ 2-methyl-3-hydroxyvalerate(2M3HV). It showed that PHAs obtained by activated sludge with different carbon sources composed of different monomer units.

Key words: polyhydroxyalkanoates; biodegradable plastic; activated sludge
CLC number: X705 **Document code**: A **Article ID**: 0529-6579(2003)S1-0118-04

1 Introduction

In the modem waste water treatment process, the effective disposal of sludge, excessively produced from the waste treatment has become a serious environmental problem. Though several methods have been proposed for the effective utilization and volume reduction of excess sludge, the major part of the excess sludge is even now disposed as a landfill without reuse. Activated sludge is considered to be a valuable biomass resource composed of a variety types and amounts of microorganisms, such as a variety of microorganisms in activated sludge is known to produce an optically active polyhydroxyalkanoates (PHAs) as an intracellular carbon and energy storage compounds under the condition of unbalance growth. The role of PHAs in the biological sewage treatment is as an intermediate metabolic product accumulated by a lot of bacteria in activated sludge as a consequence of anaerobic uptake of organic substrates under the condition of limiting nutrients (such as anoxic, high C/N and so on). Another important aspect of PHAs is as thermoplastic polyester with the remarkable characteristics of biodegradability[1,2], having the general structural formula shown in Fig. 1. When these two aspects of PHAs, as an intermediate of wastewater treatment and as a biodegradable plastic were combined, the method of production of PHAs by activated sludge was obtained, which is a new synthesis pathway to PHAs, and a popular point of sludge reduction of biological sewage disposal.

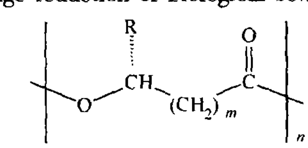

Fig. 1 General structure of PHAs

The possibility of PHAs production by activated sludge has been already demonstrated[3-5]. Satoh et al in the University of Tokyo have investigated PHAs production by activated sludge, focusing on increasing the PHA content of activated sludge. They indroduced the "microaerophilic-aaerobic" process, where a limited amount of oxygen is supplied into the anaerobic zone of the anaerobic-aerobic process. Activated sludge acclimatized in the microaerophilic-aerobic process accumulated PHA of as much as 62% of sludge dry weight[3]. But few study on the relation between carbon source and structure of PHAs was reported. In the paper, effect of carbon source on the structure of PHAs

* Received 10 December 2002; Accepted 18 April 2003
Corresponding author: dongenlin@21cn.com(LIN D E)

synthesized by activated sludge was investigated. The carbon sources used in the study were acetate (Ⅰ) and propionate (Ⅱ).

2 Experiment

2.1 Materials

The methods of activated sludge assimilation and cultivation was described in literature[4]. In brief, the seed sludge obtained from ZHUJIANG BEER PLANT in Guangzhou city was assimilated for about 2 months in a laboratory-scale anaerobic continuous culture apparatus. The cultivating medium used at this stage was artificial waste water containing $(NH_4)_2SO_4$ of 72 mg/L, $MgSO_4$ of 89 mg/L, $CaCl_2$ of 17 mg/L, KCl of 70 mg/L, K_2HPO_4 of 60 mg/L, pepton of 150 mg/L, yeast extract of 33 mg/L, and the carbon sources of 1 g/L was the two kinds of acetate (Ⅰ) and propionate (Ⅱ), respectively. At the end of the assimilation, the assimilated activated sludge was cultivated in the anaerobic process with the two different kinds of substrates as the sole carbon source. From these sludge, 2 kinds of PHAs samples were extracted by hypochloride-chloroform method. The two kinds of PHAs samples were named sample (Ⅰ) and sample (Ⅱ).

2.2 Methods

The monomer compositions of PHAs samples were determined by ^1H NMR, ^{13}C NMR, GC-MS.

2.2.1 ^1H NMR analytical procedures

^1H NMR spectra of PHAs samples were recorded on a Bruker DRX-400NMR spectrometer at 400 MHz in $w = 2\%$ solution in deuterated chloroform. The sweep width amounted to δ 12 and the corresponding data points were 8 k. A pulse angle was 90°. The ^1H NMR spectra were obtained after accumulation of 32 times. The ^1H chemical shifts were referenced to tetramethylsilane, and indicated.

2.2.2 ^{13}C NMR analytical procedures

The 100 MHz ^{13}C NMR spectra were recorded at 27 ℃ on a $CDCl_3$ solution of the polymer ($w = 2\%$) with 5.0 s pulse repetition. The sweep width amounted to δ 250 and the corresponding data points were 64 k. A pulse angle was 90°.

2.2.3 GC-MS analytical procedures

A weighed amount (about 10 mg) of PHAs was combined with 2 mL of acidified methanol (3% H_2SO_4), containing benzoic acid as the internal standard and 2 mL chloroform, heated for 4 h at 100 ℃ in Pyrex test tubes (15 mL volume) with Teflen-lined caps. Cooling, added 1 mL of distilled water, after 5 min of vigorous shaking and centrifugation, the chloroform phase containing the 3-hydroxyalkyl carboxy acid methyl esters was transferred to a GC vial for split injection of 1 μL sample into a HP6890 GC equipped with a GC column (length, 30 m; inside diameter, 250 μm; coated with 0.25 μm of phenyl methyl siloxane). The followed temperature profile was used: 2 min at 100 ℃, following by a temperature increase rate of 10 ℃/min to 200 ℃, and followed by a temperature increase 20 ℃/min to 250 ℃, 1 min at 250 ℃. The temperature of the injection point was set at 260 ℃, and the interface temperature of GC to MS was set at 260 ℃, too. The sweep width in the MS amounted to 20 ~ 550.

3 Results and discussion

3.1 Structure analysis for PHAs production by activated sludge with acetate as the sole carbon source

The activated sludge cultivation in the medium containing acetate as the sole carbon source accumulated PHAs of $w = 12.32\%$ based on the dried sludge, which is sample Ⅰ. The values M_n of M_w/M_n of the PHA sample Ⅰ, were 6.6×10^4 and 1.25, respectively (determined by GPC). Fig. 2 shows the ^1H NMR spectrum of PHA sample Ⅰ and sample Ⅱ.

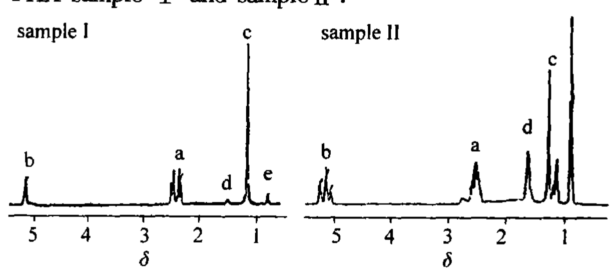

Fig.2 ^1H NMR spectrum of PHAs by sludge synthesized with acetate or propionate as the sole carbon sources

^1H NMR spectrum of sample Ⅰ has five peak, just like the ^1H NMR spectrum of P(3HB-co-3HV) in the literature[6]. The structure of 3HB-co-3HV showed in Fig. 3. From the ^1H NMR spectrum of PHA sample Ⅰ, it shows that the main monomer unit of PHA sample Ⅰ synthesized by activated sludge in the media of acetate is 3HB, mole fraction of 3HB is $x = 93.91\%$ (calculated from the peak area), and the mole fraction of 3HV is 6.09%.

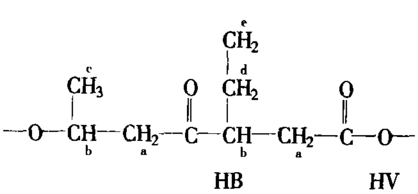

Fig.3 The structures of (3HB-co-3HV)

It is easy to understand that after acetate had been absorbed by sludge microorganism, acetate was converted to acetyl-CoA, two acetyl-CoA moieties are condensed to acetoacetyl-CoA by β-ketothiolase. A NADPH-dependent reductase reduces acetoacetyl-CoA to D-(−) 3-hydroxybutyryl-CoA, which is the linked to the growing chain of P(3HB) by the PHA synthase. As to the origin of 3HV monomer unit, it isn't clear by now. We guess the activated sludge which we used in the experiment maybe contained carbon source of odd number carbon, so the carbon source of odd number carbon would be converted to propionyl-CoA by microorganism via β-oxidation pathway.

3.2 Structure analysis for PHAs production by activated sludge with propionate as the sole carbon source

The activated sludge cultivated in the medium containing propionate Ⅱ as the sole carbon source accumulated w (PHAs) = 9.55% base on the dried sludge, which is sample Ⅱ. The values M_n and M_w/M_n of the PHA sample Ⅱ, were 5.56×10^4 and 1.37, respectively (determined by GPC). ^{1}H NMR spectrum of sample Ⅱ showed on Fig.2, and it also contained five peak of 3HB-co-3HV, but a few small peaks appear on the ^{1}H NMR spectrum, which indicated that the monomer unit of sample Ⅱ have other monomer except for 3HB and 3HV.

Total ion chromatogram of sample Ⅱ showed in Fig.4, the identity of the peaks was established by MS.

From Fig.4, it showed that sample Ⅱ mole fractions of four monomer units, namely, 28.66% 3HB, 63.13% 3HV, 2.55% 2M3HB, 5.66% 2M3HV), which have been further confirmed by ^{13}C NMR in Fig.5.

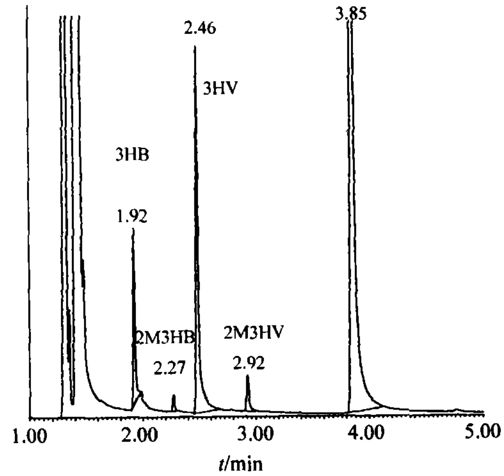

Fig.4 Total ion chromatogram of sample Ⅱ production by activated sludge with propionate as carbon source

When carbon source was propionate, PHA sample Ⅱ composed of 4 types of units, 3HB, 3HV, 2M3HB, 2M3HV. We investigated the relationship between the carbon source and the structures of PHAs. When the propionate was absorbed by the microorganisms, it was easily converted to propionyl-CoA. Acetyl-CoA would be obtained by several pathway such as intracellular glucose degradation, protein degradation, and so on. So there were two types synthesis procursor in the cell, acetyl-CoA and propionyl-CoA, which would build 3HB, 3HV, 2M3HB, 2M3HV. The scheme of synthesis pathway are shown in Fig.6.

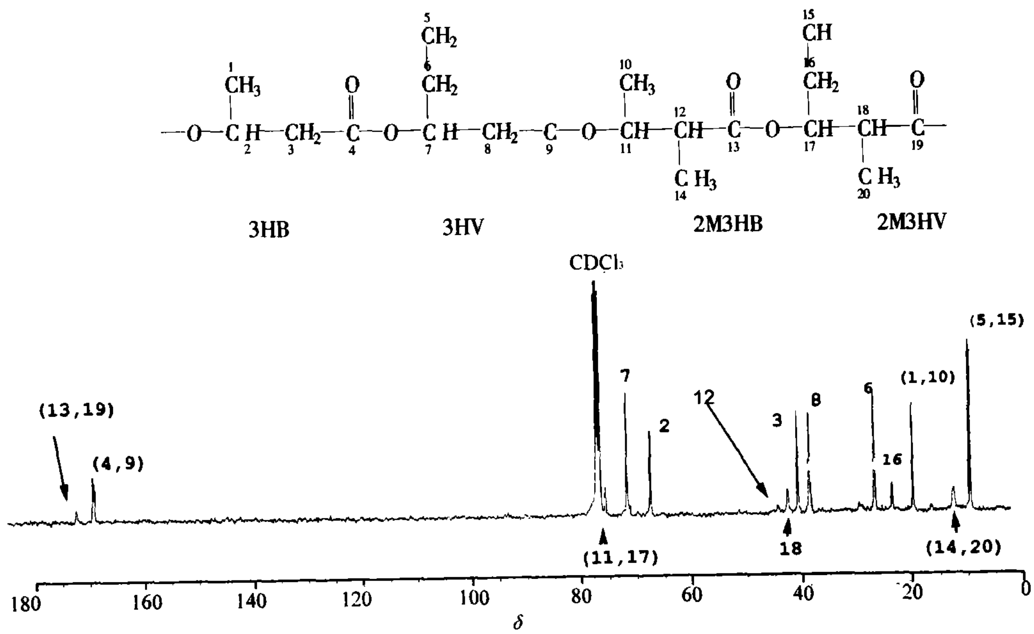

Fig.5 ^{13}C NMR of sample Ⅱ production by activated sludge with propionate as carbon source

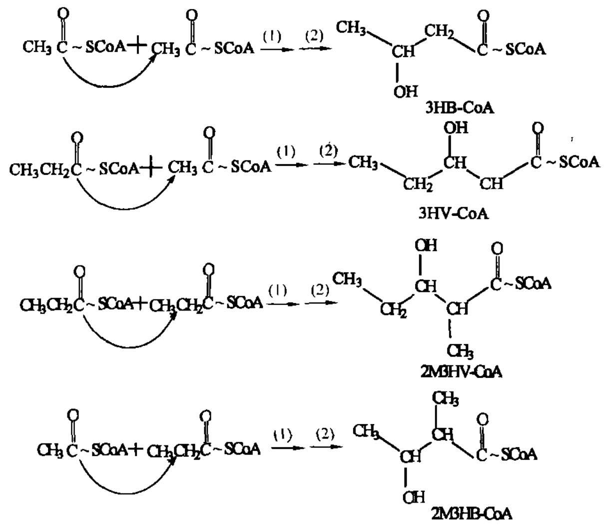

Fig.6 Scheme of synthesis pathway of 3-hydroxyalkanoate units by acetyl-CoA and propionyl-CoA
(1) condensation; (2) reduction

4 Conclusion

In the present paper, the structure of bacterially synthesized PHAs, isolated from the sludge developed in the anaerobic waste-water treatment process containing acetate and propionate as the sole carbon source, were determined by ^{1}H NMR, ^{13}CNMR, GC-MS. When the carbon source was acetate, the unit mole fraction of PHAs was 93.91% 3HB and 6.09% 3HV; When the carbon source was propionate, the mole fraction of PHAs was 28.66% 3HB, 63.13% 3HV, 2.55% 2M3HB and 5.66% 2M3HV. We could not go into the details of the biochemical mechanism in this research, but we are sure that we have showed that PHAs obtained by activated sludge with different carbon sources composed of different monomer units, which would be expected as new biodegradable materials.

Acknowledgment: This study was supported by the Natural Science Foundation (foundation item: 50278036)

References:

[1] Lee S Y. Bacterial polydroxyalkanoates[J]. *Biotechnol Bioeng*, 1996, 49(1): 1.

[2] Anderson A J, Dawes E A. Occurrence metabolism, metabolic role and industrial uses of bacterial polyhydroxyalkanoates[J]. *Microbiol Rev*, 1990, 54(4): 450.

[3] Satoh H, Iwamoto Y, Matsuo T. Activated sludge as a possible source of biodegradable plastic[J]. *Wat Sci Tech*, 1998, 38(2): 103.

[4] Lin D E. Polyhydroxybutyrate production by activated sludge in the aerobic-anaerobic process[J]. *J South China University of Technology (Natural Science Edition)*, 2002, 30(9): 100.

[5] Lin D E. Studies on biodegradable plastic-polydroxy-alkanoates production by activated sludge[D]. Gunagzhou: South China University of Technology, 2002.

[6] Doi Y, Kunioka M, Nakamura Y, et al. Nuclear magnetic resonance studies on poly (β-hydroxybutyrate) and a copolyester of β-hydroxybutyrate and β-hydroxyvalerate isolated from *Alcaligenes euteophus* H16 [J]. *Macromolecules*, 1986, 19(11): 2860.

以乙酸钠、丙酸钠为碳源的活性污泥合成聚羟基脂肪酸酯的结构分析

林东恩[1], 张逸伟[1], 孙东成[1], 沈家瑞[2]

(1.华南理工大学应用化学系,广东 广州 510641;
2.华南理工大学材料学院,广东 广州 510641)

摘 要: 聚羟基脂肪酸酯(Polyhydroxyalkanoates, PHAs)是临时储藏于活性污泥的微生物体内的能量和碳源聚合物,是完全可生物降解的热塑性塑料。为了研究碳源对PHAs结构的影响,本文采用乙酸钠、丙酸钠为碳源在厌氧条件下培养活性污泥得到2种不同的PHAs样品,通过^{1}H NMR、^{13}C NMR和GC-MS谱图确定其组成和比例:以乙酸钠培养活性污泥得到PHAs样品的单体组成以3-羟基丁酸酯(3-hydroxybutyrate, 3HB)为主,x(3HB)/x(3HV)(3-hydroxyvalerate, 3-羟基戊酸酯)为93.91:6.09;以丙酸钠培养活性污泥得到PHAs样品的单体组成主要以3HV为主,除3HB、3HV外,另外还有2-甲基-3-羟基丁酸酯(2-methyl-3-hydroxybutyrate, 2M3HB)和2-甲基-3-羟基戊酸酯(2-methyl-3-hydroxyvalerate, 2M3HV),3HB/3HV/2M3HB/2M3HV的摩尔比为28.66:63.13:2.55:5.66。由此可见采用不同碳源培养活性污泥所得到的PHAs的单体组成是不同的。

关键词: 聚羟基脂肪酸酯;可生物降解塑料;活性污泥
中图分类号: X705 **文献标识码:** A