CONVERSION OF FOOD INDUSTRIAL WASTES INTO BIOPLASTICS WITH MUNICIPAL ACTIVATED SLUDGE

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SUMMARY

Plastics have become an integral part of our contemporary life because of many desirable properties including durability and resistance to degradation. However, these non-degradable, petrochemicals-derived plastics accumulate in the environment at a rate of 25 million tons per year. Recently there is an interest in the development of a class of microbially produced bioplastics, e.g., polyhydroxyalkanoates (PHAs) which retain the desired physical and chemical properties of conventional synthetic plastics. Broader usage of biodegradable plastics in packaging and disposable products as a solution to the environmental problem would heavily depend on further reduction of costs and the discovery of novel biodegradable plastics with improved properties. In this paper, the microbial production of PHAs by activated sludge utilizing food industrial wastes is reported. The melting points of the products as well as the co-polymer composition of the products investigated by GC and NMR were compared. By use of activated sludge to convert the carbon source into PHAs not only environment-friendly bioplastics are produce, but also part of the problem of the disposal of municipal activated sludge is solved. The selection of food industrial waste as carbon resource can also further reduce the cost of production of PHAs.

INTRODUCTION

Plastics have become an integral part of our contemporary life because of many desirable properties including durability and resistance to degradation. These non-degradable, petrochemicals-derived plastics accumulate in the environment at a rate of 25 million tons per year. Several hundred thousands tones of plastics are discarded into marine environments each year causing the death of nearly one million marine animals[1]. Recently, the problems concerning with the global environment and solid waste management have created much interest in the development of biodegradable plastics. Some biodegradable plastics under

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extensive investigation are polyhydroxyalkanoates (PHA) which retain the desired physical and chemical properties of conventional synthetic plastics[2]. Poly-β-hydroxybutyrate (PHB), the most widespread and thoroughly characterized PHA, was first discovered in 1926 by M. Lemoigne as a granular inclusions (serving as energy and carbon reservoir) of the bacterium *Bacillus megaterium*. Many products can be derived from PHB, including biodegradable bottles, razors and food trays, drug release carriers, and surgical sutures[3]. A number of bacteria, including *Alcaligenes spp.*, *Pseudomonas spp.*, and a number of filamentous genera[4], and recombinant *Escherichia coli* [5] accumulate these PHAs when they encounter unfavorable nutrient-deficient conditions. The critical factor limiting the usage of PHA plastics is its high cost of production. It has been calculated that 3 tons of glucose, the feedstock used by the Imperial Chemical Industries (ICI) for commercial PHA production, must be used for each ton of polymer produced. Propionic acid which has been used as a co-substrate for the production of the copolymer poly-(3-hydroxybytyrate-co-3-hydroxyvalerate) with better physical properties than PHB is about twice as expensive as glucose[6]. Despite the numerous efforts in optimizing the PHA production process and reduction in operation costs, the current cost of PHA is still around six times as higher as that of conventional plastics[7]. Broader usage of biodegradable plastics in packaging and disposable products as a solution to environmental problem would heavily depend on further reduction of costs and the discovery of novel biodegradable plastics with improved properties. The term "activated sludge" was coined by Arden and Lockett[8] in 1914 to designate a highly active, acclimatised microbial biomass. The use of activated sludge[9, 10, 11,12] to convert nutrients into PHAs not only lead to bioplastics production, but also can solve part of the problem of the disposal of municipal activated sludge. In this paper, the microbial production of PHAs by activated sludge utilizing various types of food industrial wastes is reported.

MATERIALS AND METHODS

Source of activated sludge

In each experiment, about 10 L of activated sludge, was collected from the Wastewater Sewage Treatment Plant in Shatin. The sludge was allowed to settle for 3 h. After that, 5 L of supernatant were descanted. The remaining liquor was mixed and 2 L were poured into the reactor and mixed with 4 L of growth phase synthetic substrate or food wastes.

Sequencing batch reactor (SBR) set-up

An SBR system with a 12 L tank reactor, an automatic timer, an air compressor, two water pumps, and a magnetic stirrer, was used to culture activated sludge as shown in Figure 1. The reactor had a working volume of about 10 L and the substrate was introduced by means of a peristaltic pump to give 0.4 L/min. An air pump was connected to provide continuous aeration to the system.

Reactor operation programme

A two stage fermentation process was employed. The first two days were the growth phase for the microorganisms so that carbon, nitrogen and phosphorous sources were introduced. The next five days was the polymer production phase and the medium was nitrogen limited. The operational mode of the SBR was set as follows:

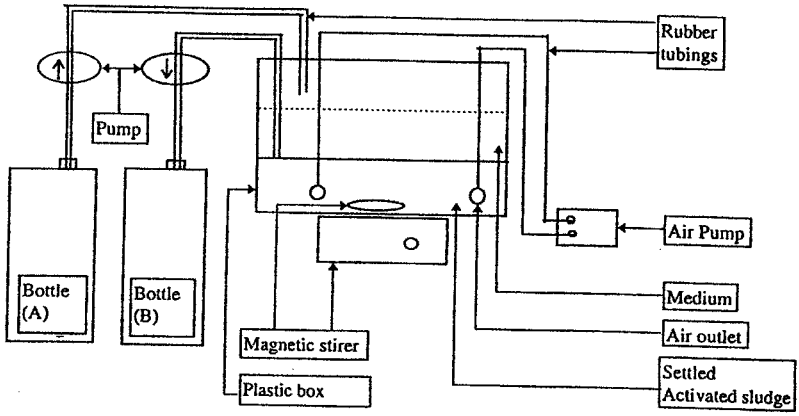

Fig. 1. A schematic diagram of the microbial production of PHA in an SBR with activated sludge

SBR operation cycle

Mode	Period
Fill	10 min
Aeration	6 h
Settle	2 h
Decant	10 min
Idle	1 h 40 min

Media

Growth phase medium

Synthetic media:

Each liter of medium consisted of 4.4 g glucose monohydrate, 0.25 g ammonium sulphate and 0.04 g KH_2PO_4

Industrial food waste media:

Soya waste from VITASOY Company, and beer waste from Carlsberg Company was used. 1.2 L of 1 M HCl was added to 1 kg soya waste or beer waste respectively. The mixture was incubated at 80°C for 8 h. The resultant mixture was centrifuged at 900 rpm for 20 minutes. Then the solid was discarded and the supernatant was neutralized to pH 7 with NaOH. The solution was diluted to 8 L before use.

Production phase media:

The composition was the same as that of growth phase medium except that no ammonium sulphate was added.

Extraction and precipitation of biopolymer

Sampling, extraction, and analytical techniques were conducted according to the previously described report[11, 13].

RESULTS AND DISCUSSION

The yields of polyhydroxybutyrate (PHB) and cell growth of activated sludge using brewery malt waste during fermentation are shown in Table 1. The accumulation of PHB and cell growth reached 6.5 g/L, and 15.1 g/L cell dry weight (CDW) after 32 h of fermentation time, respectively. Cell growth went through a fast, and then a slower stage after 10 h of fermentation before reaching another fast stage after 28 h. However, polymer accumulation was started at a very early stage of fermentation and reached a high production level after 30 h of fermentation. The results showed that activated sludge microorganisms produce polymers without going through an initial cell growth stage.

Table 1. Cell growth (CDW) and polymer (PHB) accumulation in microorganism from activated sludge using brewery malt waste as carbon source.

Fermentation time in h	g/L		Fermentation time in h	g/L	
	PHB	CDW		PHB	CDW
0	0.150	0.500	20	1.650	6.800
2	0.175	0.575	21	2.025	6.950
3	0.525	1.700	23.3	2.050	7.050
4	0.575	1.950	24	2.200	7.250
5	0.650	3.025	25	2.350	7.300
6	0.625	3.025	26	2.500	7.500
7	0.750	3.575	27	2.700	7.800
8	0.800	4.450	28	3.000	7.825
9	0.850	5.550	29.4	3.125	8.125
11	0.975	6.250	30	3.400	9.050
13	1.000	6.350	31	3.875	9.875
15	1.300	6.375	31.5	4.200	10.300
18.6	1.525	6.700	32	6.550	15.125

The production of PHA by activated sludge using fructose (C:N ratio of 16:1 and of 96:1), glucose, soya waste, and brewery malt waste were investigated and characterized by gas chromatography and ^{1}H-NMR studies as shown in Figures 2, 3, 4, and Figure 5. In Figure 2, the GC shows 3HB and 3HV with a peak area ratio of 1: 4. Thus, using fructose (C:N = 16:1) as a carbon source, the copolymer P(3HB-co-3HV) is produced with carbon ratio 3HB:3HV of 1: 4. Changing the C:N ratio of fructose to 96:1 did not affect the result of the copolymer composition (Figure 3). The presence of 3HB and 3HV is verified by ^{1}H-NMR analysis as displayed in Figures 2 and 3. The result of the fermentation of glucose (Figure 4) by activated sludge shows 3HB and 3HV in a GC peak area ratio of 55: 45. The production of polymers by activated sludge using soya waste also yielded PHB and PHV with a peak area ratio of 3:1, as illustrated in Figure 5. The ^{1}H-NMR spectrum of the polymers produced with activated sludge using soya waste verified the presence of 3HB and 3HV units.

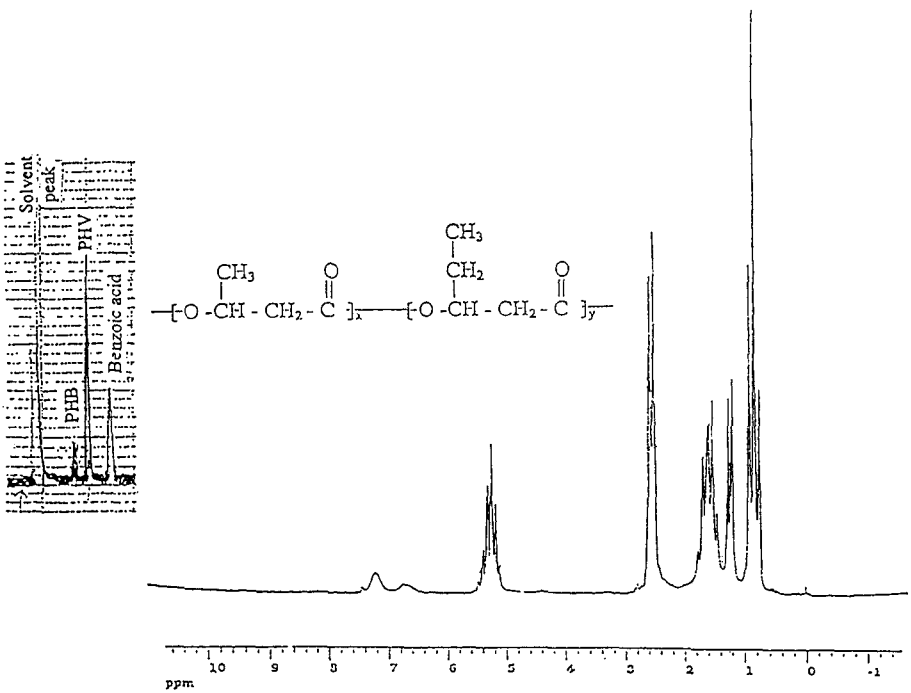

Fig.2. GC and ^{1}H-NMR analysis of microbial PHA production with activated sludge using fructose (C: N = 16: 1) as carbon source.

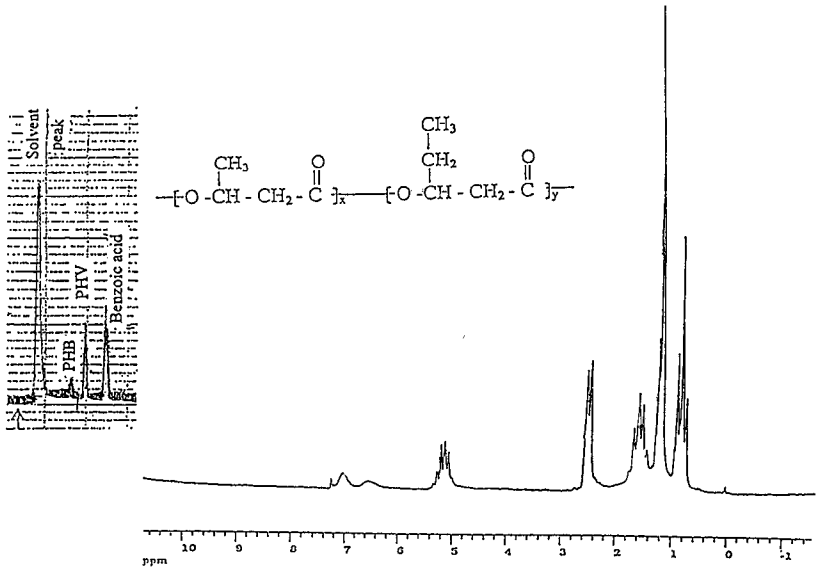

Fig. 3. GC and ^{1}H-NMR analysis of microbial PHA production with activated sludge using fructose (C: N = 96: 1) as carbon source.

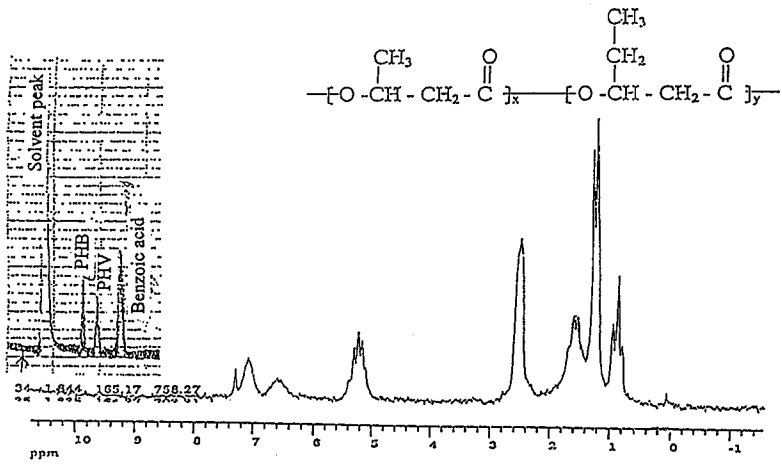

Fig. 4. GC and ^{1}H-NMR analysis of microbial PHA production with activated sludge using glucose (C: N = 16: 1) as carbon source.

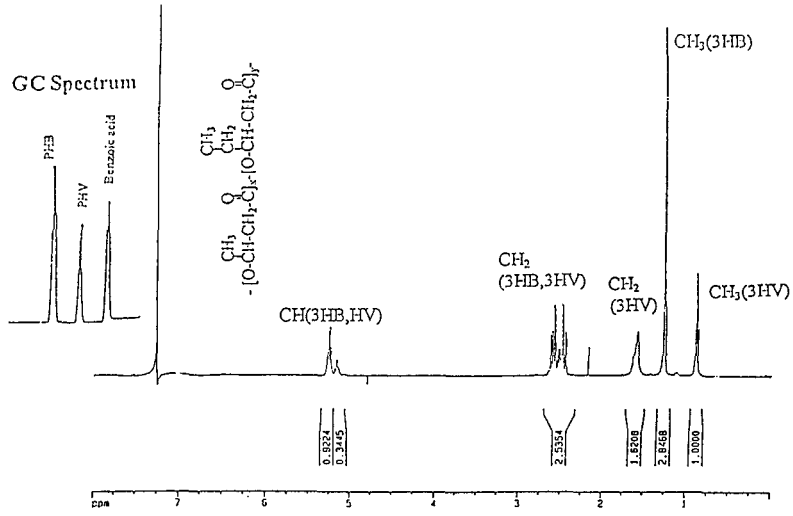

Fig. 5. ^{1}H-NMR analysis of microbial PHA production with activated sludge using soya waste as carbon source.

Brewery malt waste was previously used in this laboratory as nutrient for the production of polymers by activated sludge[13], and the results indicated that > 90% of the polymer produced was PHB. This observation is in agreement with the microbial production of polymer by *Alcaligenes latus* using brewery malt waste as carbon source[14]. The reason for the findings that different copolymers could be formed by activated sludge using different sources of industrial food wastes could be due to the fact that the activated sludge contains a wide variety of bacterial cultures, where many different species of which could produce PHA. Different types of PHA synthetase with different substrate specificity may be present in the mixed bacterial culture in the activated sludge. In beer and soya waste a wide variety of sugars as carbon source is present (e.g. maltose, sucrose, fructose, etc.). Our data indicated that the yield of PHA produced using soya waste as nutrient medium is higher than using synthetic nutrient media containing glucose as carbon source.

The melting points of PHB and PHV were found to be 177.0°C, and 100°C, respectively, as shown in Table 2, and these values are close to the previously reported values[13]. The melting point of PHA produced from activated sludge using soya waste was found to be 165.2°C, a melting point between those of PHB and PHV, and the reason for the lower melting

temperature as compared with PHB could be due to the fact that the biopolymer is a copolymer as demonstrated in Figure 5. This explanation can be applied also to the observed melting points of the polymers produced by activated sludge using fructose and glucose as nutrients. From previously published report[15], the PHB/HV copolymer with 10 % of HV has a melting point of 150.0°C. The melting suppression observed for polymers produced with activated sludge using malt waste could be due to the presence of 3HV units.

Table 2. Melting point of PHA standards and PHA produced by activated sludge using industrial food wastes

Sample	Polymer Composition	Melting Point (°C)
Standard PHB	PHB	177
Standard PHV	PHV	100
PHA produced by using soya waste	P(3HB-co-3HV)	165
PHA produced by using fructose	P(3HB-co-3HV)	142(C:N = 16:1) 144 (C:N = 64:1)
PHA produced by using glucose	P(3HB-co-3HV)	179
PHA produced by using malt waste	P(3HB-co-3HV)	157

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