

PRODUCTION OF BIODEGRADABLE THERMOPLASTIC MATERIALS FROM ORGANIC WASTES

FIELD OF THE INVENTION

5 [0001] The present invention relates to systems and processes for producing biodegradable thermoplastic materials from various organic wastes.

BACKGROUND OF THE INVENTION

10 [0002] Polyhydroxyalkanoates (PHAs) are polyesters accumulated by many prokaryotic organisms as intracellular carbon and energy reserve materials under limited nutrients, such as nitrogen or phosphorous. PHA polymers demonstrate thermoplastic or elastic properties depending upon their particular composition. These materials have attracted interest because of their potential use as biodegradable alternatives to petroleum-based synthetic plastics such as polypropylene and polyethylene. Efforts have been made to produce PHAs by microbial fermentation on glucose and organic acids. The
15 production of biodegradable thermoplastics from organic wastes can provide multiple benefits to the environment, and contribute to sustainable development.

20 [0003] Organic wastes are usually complex in nature, and cannot be directly utilized by PHA-producing microbes such as *Ralstonia eutropha*, a representative bacterium for PHA synthesis. Hydrolysis and acidogenesis is the first step in converting the wastes to short-chain fatty acids such as acetic, propionic and butyric acids, that can be further utilized by PHA-producing bacteria. At present, there are technological difficulties in coupling the waste acidogenesis and PHA synthesis steps. In essence, both acid-producing and PHA-producing cells can be cultivated in a mixed culture, and the acids released by the former are directly utilized by the latter. The PHA content of the
25 solid mass, however, is not high enough (10-30 % by wt.) for effective polymer recovery, due to the considerable amount of non-biodegradable matter in the waste, as well as the biomass of non-PHA producing microbes. Furthermore, a high acid concentration due to the high microbial activity of acidogenic microbes would inhibit the microbial activity of PHA-producing cells.

[0010] FIG. 5 is a graph of the mole percentage of hydroxyvalerate (HV) monomer in the copolymer of poly(hydroxybutyrate-co-hydroxyvalerate) synthesized from the fermentative acids transferred from the dialysis membrane;

[0011] FIG. 6 is a graph of the specific PHA synthetic rates and specific cell growth rates with the dense membrane and the dialysis membrane;

[0012] FIG. 7 are graphs of time courses of a batch culture of *R. eutropha* on a binary mixture of pure butyric acid and lactic acid (a: the acid concentrations and the specific consumption rates; b: growth of *R. eutropha* and PHA synthesis on the mixed acids).

10 DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0013] The present invention relates to a method and system for converting organic wastes to biodegradable polyhydroxyalkanoates (PHAs). The method comprises (a) treating the organic wastes with an acidogenic microbial population to form volatile organic acids; and (b) polymerization of the volatile organic acids by PHA-producing microbial species to form PHAs. The particular PHAs may comprise either short chain (3 to 5 carbon) monomers or long chain (6 to 14 carbon) monomers. The PHA-producing microbial species may be, e.g., *R. eutropha* or *P. oleovorans*. The viscosity-average molecular weight (M_v) of PHA polymers ranges from 50 to 100 kD.

[0014] The system for converting organic wastes to biodegradable PHAs comprises: (a) a first compartment, for acidogenesis of organic wastes without oxygen; and (b) a second compartment, for polymer synthesis by enriched cultures of *R. eutropha*, *P. oleovorans*, or mixtures thereof with oxygen. A barrier facility couples the first and second compartments. The compartments are characteristically bioreactors.

[0015] The present invention will be further described by the following examples.

25 Examples

(MWCO) of 6,000-8,000 (flat width 10 mm, 28 μ m thickness and 0.23 m in length). The PHA-synthesis reactor was maintained at 30 °C via a water jacket and pH 7.5 by adding 3 M HCl or 3 M NaOH solution. The dissolved oxygen concentration was maintained at about 20% of air saturation with 3-vvm aeration. Further in FIG. 1, there is provided
5 temperature control by the water bath 14.

[0019] PHA production from a mixture of pure butyric and lactic acids was conducted, as a control in a 2 L fermenter equipped with two six-bladed disk turbines. About 2.5 g cell mass of *R. eutropha* harvested from a nutrient-rich culture was re-suspended in 1.5 L mineral solution. A mixture of butyric acid (ca. 4 g/L) and lactic acid
10 (ca. 4 g/L) was added into the mineral solution as the sole carbon source for PHA synthesis. The dissolved oxygen concentration was maintained above 20% air saturation with 1-vvm aeration and an agitation speed of 600 rpm. The cultivation temperature was controlled at 30 °C and the pH was controlled at 7.5 by adding 3 M HCl or 3 M NaOH solution. Cell growth was monitored by measuring the optical density (OD) at 620 nm.

[0020] Dry cell weight and PHA concentration were determined as follows:
Thirty milliliters of culture broth was centrifuged at 10,000 g for 10 min and lyophilized to constant weight. PHA was extracted from the lyophilized cells in excess chloroform (10 mL chloroform for 0.2 g cell biomass) at 60 °C for 24 hours. The residual solid was removed by filtration through a glass filter and defined as the residual biomass. A PHA
20 film was formed and its mass was weighed after the chloroform was completely vaporized at room temperature. The PHA content was defined as the percentage of the PHA mass in the dry cell mass. Acetic acid, propionic acid and butyric acid were measured by gas chromatography equipped with a flame ionization detector by direct injection of acidified aqueous samples (pH 2 to 3) into a fused silica capillary column
25 (ϕ 0.25 mmx25 m, Supelco, USA). Lactic acid was measured by high performance liquid chromatography equipped with a C₁₈ column and a UV Diode-array detector at 210 nm. The mobile phase was a 0.2% H₃PO₄ solution. The composition of PHA was determined after methanol esterification by GC-mass spectroscopy, a method developed by Lageveen et al.

[0025] PHA production coupled with anaerobic digestion of food scraps was conducted in the air-bubbling reactor 11 with two types of barriers; dense phase membrane and dialysis membrane. FIG. 3 shows the time courses of PHA production when the dense phase membrane was used for acid transfer from the acidic slurry. The residual biomass was increased from 1.9 g/L to 4.5 g/L in 73 hours. The PHA content and PHA concentration reached 60.2% and 6.5 g/L in 70 hours, respectively (FIG. 3a). This means that the acids in the acidic slurry were transferred to the cells of *R. eutropha* through the silicone dense phase membrane and used as the sole carbon source for cell growth as well as PHA accumulation. Acetic, propionic and butyric acids were detected in the culture broth of *R. eutropha* at different times, which depended on their relative rates of mass transfer through the membrane and consumption by *R. eutropha* of individual acids (FIG. 3b). Butyric acid was the major fermentative acid and its concentration was below 120 mg/L in the mineral solution. Its transfer rate was the fastest due to its highest concentration difference across the barrier. Acetic acid and propionic acid appeared in the late stage, and their concentrations reached 3.0 mg/L and 1.6 mg/L, respectively. Compared with the acid concentrations in the acidic slurry, the acid concentration differences across the dense phase membrane were 27.4 g/L (butyric acid), 5.5 g/L (acetic acid), and 1.8 g/L (propionic acid), respectively. No lactic acid was detected in the PHA-producing reactor even though its concentration difference across the membrane was up to 32.6 g/L.

[0026] FIG. 4 shows the PHA production when the dialysis membrane was used as a barrier between acidogenesis and PHA production. Both acetic acid and butyric acid were detected in the early stage in the PHA-producing reactor, while lactic acid and propionic acid were detected after 40 hours. Different from FIG. 3b, lactic acid was also transferred through the dialysis membrane in addition to the three volatile fatty acids. Moreover, the concentrations of four acids in the PHA-producing reactor (11 in FIG. 1) were much higher than those from the dense phase membrane. After 60 hours, the acid concentrations rose quickly because of the slowdown of PHA synthesis. The concentration of acetic, propionic, butyric and lactic acids in the broth of polymer synthesis reached 0.42, 0.16, 1.40 and 1.51 g/L, respectively (FIG. 4a). With a large

Table 1. Enzyme activities at different pH levels during anaerobic digestion of food scraps

pH	6.8	6.5	6.0	5.7	5.3	5.0
Amylase activity (u/L)	84.0	77.3	70.5	54.8	30.4	17.3
Lipase activity (u/L)	38.9	37.4	36.3	35.7	23.1	13.9
Protease activity (u/L)	10.7	12.4	24.1	15.2	22.1	20.5

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[0029] Amylase and lipase showed high activities at neutral pH levels; protease showed high activity at low pH levels. This points to the fact that starch and oily matter in food scraps were quickly hydrolyzed at neutral pH, and protein hydrolysis was accelerated at low pH, releasing amino acids as nutrients. A pH swing between the neutral and acidic levels, therefore, enhances the food scrap digestion, compared to a constant pH level. In the first 14 days, most of the easily digestible solids had been used and the microbial activity declined. The enzymatic activities even at a neutral pH level became low (10-15 u/L), compared to 40-80 u/L at the beginning of anaerobic digestion.

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[0030] Acid mass transfer from the acidic slurry to the PHA synthesis medium was essential to the coupling of the two reactors. The mass transfer rates of organic acids affected not only the PHA synthesis but also the food scrap digestion, because the pH could be controlled with continuous removal of the organic acids. PHA production is dependent on the carbon source provided, and, to some extent, more PHA could be synthesized with more acids being transferred from the acidic reactor at a faster rate. The continuous removal of the accumulated acids in the acidic slurry could also raise the enzyme activity such as amylase and lipase for hydrolysis of food scraps.

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[0031] The mass transfer of the small molecules of organic acids relies on molecular diffusion in the barriers, and hence, the barrier structure has a significant effect

barriers. Acids in water and in slurry had similar dialysis rates (No. 2 & No. 4 for the dense phase membranes and No. 5 & No. 6 for the dialysis membrane.)

Table 2. Dialysis rates of organic acids in two media through two dense phase membranes and one dialysis membrane

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No.	Membranes/media	Acid dialysis rates (mmol/sec/m ²)			
		Acetic acid	Propionic acid	Butyric acid	Lactic acid
1	Dense membrane ^A (3.2 mm thickness)	1.05E-5	1.14E-5	2.96E-4	0
2	Dense membrane ^A (1.6 mm thickness)	1.94E-5	2.25E-5	6.16E-4	0
3	Dense membrane ^B (1.6 mm thickness)	9.72E-5	1.24E-4	6.07E-4	0
4	Dense membrane ^C (1.6 mm thickness)	2.01E-5	2.14E-5	6.21E-4	0
5	Dialysis membrane ^A	2.52E-2	1.07E-2	0.135	0.147
6	Dialysis membrane ^C	2.61E-2	9.59E-3	0.140	0.154

Liquid media:

10 A: Water solution (pH 5), and acetic, propionic, butyric and lactic acids are 5, 2, 25 and 30 g/L, respectively.

B: Water solution (pH 5), and acetic, propionic, butyric and lactic acids are 25 g/L, respectively.

15 C: Food waste slurry (pH 5), and acetic, propionic, butyric and lactic acids are 5.45, 1.84, 25.7 and 31.6 g/L, respectively.

[0032] Individual organic acids including acetic acid, propionic acid, butyric acid
20 and lactic acid have been used by *R. eutropha* as the carbon source for PHA production. From the anaerobic digestion of organic wastes, a mixture of fermentative acids was usually obtained. Furthermore, the composition of acid mixture in the polymer-producing reactor for PHA synthesis was determined by the types of barriers used for reactor coupling. With the dense phase membrane, butyric acid was the major acid transferred,

[0034] By utilizing the system and process of the present invention, organic acids are continuously released from anaerobic digestion of food scraps and transferred across a barrier for PHA synthesis. Because there are no solid mixing and hydraulic washout of cells, an enriched culture of PHA synthesis can be maintained to produce a biomass of very high PHA content. The 72.6% (w/w) PHA achieved is a high polymer content obtained from organic waste treatment and comparable to the polymer content obtained from pure glucose fermentation. The high polymer content is the prerequisite to develop a downstream process for cost-effective polymer recovery from cell mass. Thus, the present invention demonstrates that organic wastes can be utilized for valuable products.

10 [0035] Table 3 shows the viscosity-average molecular weight (M_v) of PHA polymers produced under controlled carbon-nitrogen weight ratios (C/N).

Table 3. Viscosity-average Molecular Weight (M_v) of PHA Biopolymers formed on Organic Acids under Initial Carbon/Nitrogen ratio (C/N)

C/N	4	9	30	45	72
M_v (kD)	820	830	730	580	510

- (a) a first compartment, for acidogenesis of organic wastes; and
 - (b) a second compartment, for polymer synthesis by enriched cultures of polyhydroxyalkanoate (PHA) producing microbial species comprising *R. eutropha*, *P. oleovorans*, or mixtures thereof,
- 5 wherein the fermentative acids are transferred from the first to the second compartments through barriers.
9. The system as recited in claim 8, wherein the barriers prevent mixing of microbial cells, particulates and water in the two compartments.
10. The system as recited in claim 8, wherein the barriers maintain different
10 culture conditions in the two compartments.