Coupling System for Food Wastes Anaerobic Digestion and Polyhydroxyalkanoates Production with Ralstonia eutropha

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Abstract: A new technology was developed to couple the anaerobic digestion of food wastes with production of polyhydroxyalkanoates (PHAs). Acetic, propionic, butyric and lactic acids were produced during food wastes anaerobic digestion and their concentrations reached 5.5, 1.8, 27.4 and 32.7 g/L, respectively under appropriate digestion conditions. The fermentative acids were transferred through a dialysis membrane to an air-lift reactor for PHA synthesis by *Ralstonia eutropha*. Dry cell concentration and PHA content reached 22.7 g/L and 72.6%, respectively. The obtained PHA was a copolymer of β-hydroxybutyrate (HB) and β-hydroxyvalerate (HV) with 2.8% (mole ratio) of HV units in polymer.

Key words: food wastes; fermentative acids; polyhydroxyalkanoates; PHAs; *Ralstonia eutropha*; anaerobic digestion

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1 INTRODUCTION

Polyhydroxyalkanoates (PHAs) are polyesters accumulated by a number of bacteria as the intracellular carbon and energy reserve materials under unbalanced growth conditions[1]. Industrial effort has been made to produce PHAs with microbial fermentation of glucose and organic acids[2]. The major obstacle to wide acceptance of PHAs is their high price, more than 10 times higher than those of synthetic plastics[3]. Novel technologies have been investigated to produce PHAs from organic wastes in wastewater[4], industrial wastes[5], and municipal wastes[6]. The organic wastes, however, are usually complicated in composition, and cannot be directly utilized by PHA-producing bacteria such as *Ralstonia eutropha*[1]. Hydrolysis and acidogenesis is the first essential step to convert the wastes to short chain volatile fatty acids such as acetic, propionic and butyric acids for PHA synthesis by *R. eutropha*[6]. There are, however, technology bottlenecks in coupling the waste acidogenesis and PHA synthesis.

In a simple and direct way, both acids-producing and PHA-producing cells can co-exist in a mixed culture, and the acids released by the former can be immediately utilized by the latter. The PHA content in the harvested solid biomass, however, is not high enough (around 30%, ω) for a cost-effective polymer recovery because of the considerable amount of non-biodegradable content in wastes and the biomass of non-PHA-producing microbes[4]. In a way of indirect coupling, the fermentative acids could be first recovered and concentrated from the diluted acidogenic solution such as by evaporation or ion exchange, then fed to *R. eutropha* for polymer synthesis[7,8]. Although the PHA content (50%~60%, ω) of cell mass from the acids is comparable to that from pure acid

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fermentation, the acid recovery cost is prohibitive because of the diluted acid concentration (<100 g/L) in a complicated digestion solution. An ideal process should have two separated bioreactors to satisfy the different physiologies and metabolic activities of two types of microbes: one for acidogenesis of organic wastes and another for an enriched culture of PHA-producing strain such as *R. eutropha*. The fermentative acids should be transferred from the first reactor to the second reactor without causing solids mixing between the two reactors.

In this study, a new strategy based on acid dialysis was designed and demonstrated to couple the bioreactors for acidogenesis of food wastes and for PHA synthesis. The food wastes were digested in the first anaerobic reactor to produce the fermentative acids that were continuously fed into the second PHA-producing reactor via a proper membrane module. An enriched culture of *R. eutropha* was successfully maintained in the second reactor, gaving a very high PHA content.

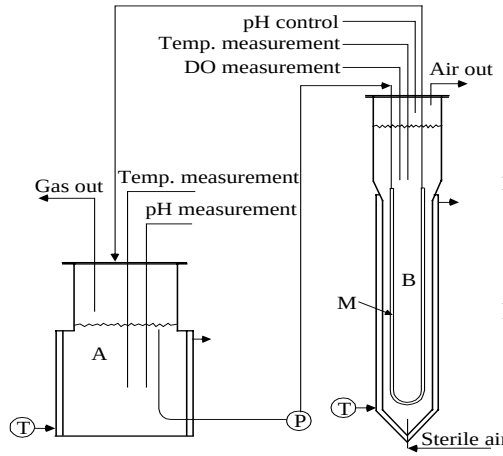
2 MATERIALS AND METHODS

2.1 Food Wastes Anaerobic Digestion at Different pH Conditions

The set-up coupling the anaerobic digestion of food wastes with PHA production is shown in Fig.1. Food wastes were collected from a canteen on campus, mixed with water in a ratio of 1:1, and crushed in a blender to slurry. A natural inoculum for food wastes digestion was prepared by keeping some food wastes in anaerobic conditions for two to three weeks. The inoculum (25%~30%, φ) was mixed with fresh food wastes and the pH was first adjusted to 7.0 with NaOH (6 mol/L). Five batches of food wastes anaerobic digestion were carried out under different pH conditions at 35°C in a 5 L reactor (reactor A) with a working volume of 3 L.

2.2 Microorganism and Growth Conditions

Ralstonia eutropha ATCC 17699 was used in this study for the production of PHA. The strain was maintained by monthly subculture on 2.0%-agar slants (pH 7.0) containing (per liter): 5 g yeast extract, 2.5 g beef extract, 5 g peptone and 5 g $(NH_4)_2SO_4$. The slants were incubated at 30°C for 24 h. The same medium without agar was used for seed culture. The seed culture was prepared in 2 L flasks containing 500 ml medium in a rotary shaker for 24 h at 200 r/min and 30°C. The culture was harvested aseptically and then transferred to a mineral solution for PHA production.



A. Food wastes digestion reactor
B. Air-lift reactor for PHA synthesis
M. Dialysis membrane
P. Peristaltic pump for acidified solution recycle
T. Temperature control

Fig.1 Set-up of the coupling system for food wastes digestion and PHA production

2.3 PHA Production Coupled with Food Wastes Anaerobic Digestion

PHA synthesis was conducted in reactor B, a 1.6 L air-lift fermenter (Bioengineering AG, Switzerland) with a working volume of 1.3 L (Fig.1). Around 3.0 g of *R. eutropha* was suspended in the mineral solution containing (per liter): 1.0 g $(NH_4)_2SO_4$, 5.8 g K_2HPO_4, 3.7 g KH_2PO_4, 0.4 g $MgSO_4$ and 1 ml of a microelement solution. The microelement solution contained the same components as described by DU et al.[9]. The acidogenic slurry in reactor A was recycled between the two reactors via a peristaltic pump (with a flow rate of 120 ml/min) and a tubular membrane module that was immersed in the mineral solution in reactor B. A dialysis tubular membrane (cellulose) with nominal MW_{CO} 6000~8000 (width 10 mm, thickness 28 μm and 0.23 m in length) was selected for the transfer of acids. The PHA-synthesis reactor was maintained at 30°C via a water jacket and pH 7.5 by adding 3 mol/L HCl or 3 mol/L NaOH solution. The dissolved oxygen concentration was kept at around 20% of air saturation at an aeration of 3.0 (volume ratio).

2.4 Analytical Procedures

Cell growth was monitored by measuring the optical density (OD) at 620 nm. Dry cell weight and PHA concentration were determined as described by DU et al.[9]. PHA content was defined as the percentage of PHA mass in dry cell mass.

Acetic, propionic and butyric acids were measured by gas chromatography (GC) with direct injection of acidified aqueous samples (pH 2 to 3) (Supelco Fused Silica Capillary column, ϕ 0.25 mm×25 m; a flame ionization detector). Lactic acid was determined by HPLC (C_{18} column, 0.2% H_3PO_4 water solution as the mobile phase, UV Diode-array Detector at 210 nm; HEWLETT PACKARD 1090 Liquid Chromatograph). The composition of PHA was determined by GC-mass spectroscopy (GC–MS) based on a method developed by Lageveen et al.[10].

3 RESULTS

3.1 Food Wastes Anaerobic Digestion under Different Conditions

Figure 2 shows the food wastes anaerobic digestion under natural pH condition. Four organic acids were detected as the fermentative products in food wastes digestion solution. Among them, lactic and butyric acids(LA, BA) were the two major acids, and their concentrations reached 16.8 and 18.1 g/L at the 15th and 14th days. Acetic and propionic acids (AA, PA) reached their maximal values of 4.41 and 1.52 g/L at the 16th and 12th days. After that, the concentrations of the four acids approached roughly their asymptotic values. The pH dropped to 4.4 in the 6th day due to the accumulation of large amount of acids, and remained almost constant afterwards. The further accumulation of acids was probably inhibited by the low pH in the early stage or by the higher concentration of accumulated acids.

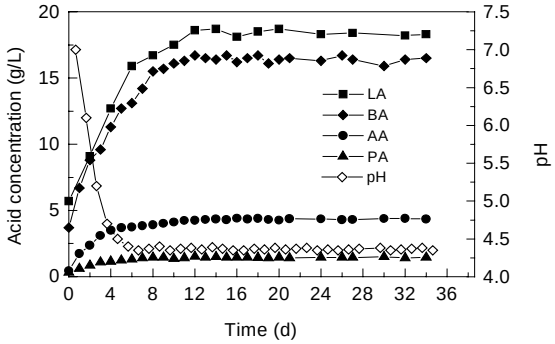


Fig.2 Food wastes anaerobic digestion under natural pH

To investigate the possible inhibitive factors, three batches of food wastes anaerobic digestion were carried out, in which the pH was adjusted to 7.0 when it dropped to 6.0, 5.5, 5.0, respectively. From the results showed in Table 1, it is clear that more propionic, butyric and lactic acids could be produced at lower pH, while more acetic acid was produced at higher pH during food wastes digestion. Furthermore, it was observed that the concentrations of the four acids decreased due to further pH adjustment in the late stage of the digestion, and neutral pH in late stage caused the consumption of acids instead of their accumulation.

Table 1 Fermentative acids produced during food wastes anaerobic digestion

pH	Acetic acid (g/L)	Propionic acid (g/L)	Butyric acid (g/L)	Lactic acid (g/L)
6.0	13.7	1.29	9.1	10.8
5.5	10.8	1.41	14.5	15.7
5.0	7.24	1.71	23.5	28.9

Note: pH was adjusted to 7.0 when it dropped to 6.0, 5.5 and 5.0, respectively

It was reported that butyric and lactic acids were the more effective carbon sources than acetic acid for cell growth and PHA production with *R. eutropha*[8, 11]. β-hydro-xyvalerate (HV) unit which could improve PHA properties was only synthesized from propionic acid[12]. For the purpose of effective PHA production in the coupling system, the food wastes should be digested under the conditions which favor the formation of butyric and lactic as well as propionic acids. From the above observations, it is obvious that, in the early stage, the pH should be adjusted to 7.0 when it dropped to around 5.0 in order to promote the decomposition of solid wastes, while it should be kept at relatively low value to avoid the reutilization of acids in the late stage.

Figure 3 shows the food wastes anaerobic digestion in which pH was adjusted to 7.0 three times when it dropped to around 5.0. Among the four fermentative acids, lactic and butyric acids were the major acids, and their concentrations increased quickly in the first 17 days and reached 32.7 and 27.4 g/L at the end of batch digestion. Around 5.5 g/L of acetic acid and 1.8 g/L of propionic acid were accumulated and kept at quite constant levels after 12 days [Fig.3(a)].

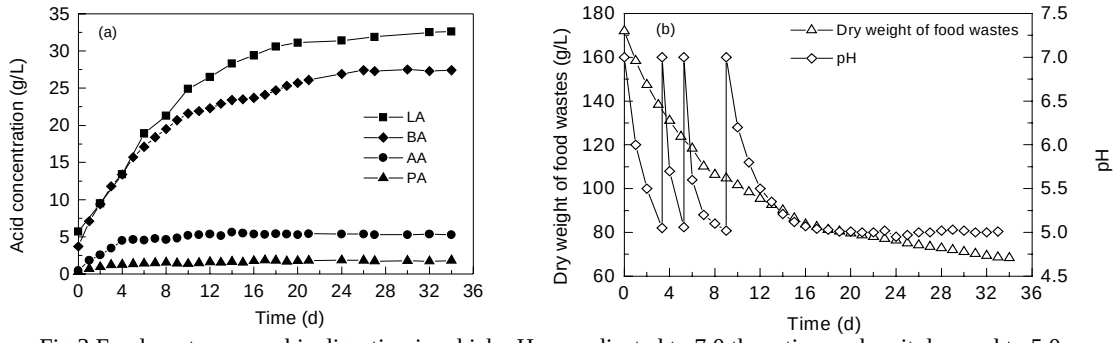


Fig.3 Food wastes anaerobic digestion in which pH was adjusted to 7.0 three times when it dropped to 5.0

The dry weight of food wastes declined quickly in the first 7 days, and more than 59.5%(ω) of the digested food wastes was consumed during this period. In the initial amount of food wastes (172.0 g/L), 103.7 g/L or 60.3% (ω) was digested in 34 days. The pH dropped rapidly from 7.0 to around 5.0 due to the accumulation of large amount of acids in the early stage and it was adjusted back to 7.0 three times in the first 10 days. After that, the pH was not adjusted because of a much

slower pH decline or acid release in the following stage. Only 13.7% of the digested food wastes was consumed in the last 17 days [Fig.3(b)]. The slowdown of solid digestion might not be attributed to pH only, the solids became more difficult to be digested.

After the concentrations of acids reached relatively high levels in 17 days, the acidic slurry was recycled through the PHA-producing reactor (reactor B) for PHA synthesis.

3.2 Organic Acids Transfer via Dialysis Membrane

Acid mass transfer from the acidic slurry to PHA synthesis medium was essential to the complying of the two reactors. PHA production is dependent on the carbon sources provided, and to some extent, more PHA could be synthesized with more acids being transferred from the acidic reactor at a faster rate. Theoretically, all the small molecules ($MW \leq MW_{CO}$) can diffuse through the dialysis membrane, and the solubility of acids in membrane had nil effect on the dialysis rate. Dialysis rates of acetic, propionic, butyric and lactic acids in food wastes slurry reached 2.61×10^{-2}, 9.59×10^{-3}, 0.140 and 0.154 mmol/(s·m^2) when the concentrations of acetic, propionic, butyric and butyric acids were 5.5, 1.8, 25.7, 31.6 g/L, respectively in digested solution. It is clear that the four fermentative acids could be transferred with dialysis membrane.

3.3 PHA Production Coupled with Food Scraps Digestion

PHA production coupled with food wastes anaerobic digestion was conducted in an air-lift reactor with dialysis membrane. Figure 4 shows the time courses of PHA production with dialysis membrane for acids transfer from the digested solution. Acetic and butyric acids were detected in the early stage in the PHA-producing reactor, while lactic and propionic acids were detected after 40 h. The concentrations of acetic, propionic, butyric and lactic acids in the culture broth accumulated to 0.42, 0.16, 1.40 and 1.51 g/L, respectively at the end of PHA fermentation [Fig.4(a)].

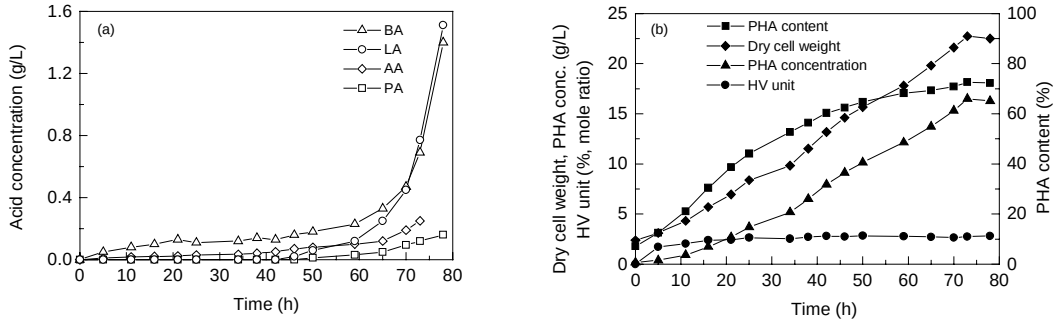


Fig.4 Time courses of PHA production with dialysis membrane

Dry cell weight, PHA content and PHA concentration increased and reached the maximal values of 22.7 g/L, 72.6% and 16.5 g/L in 73 h, respectively [Fig.4(b)]. It seemed that the acids in the acidic slurry were transferred to the PHA synthesis reactor via dialysis membrane and could be used as the carbon sources by *R. eutropha* for cell growth and PHA accumulation. HV unit, synthesized from propionic acid, was detected in PHA and its mole fraction reached around 2.8% [mole ratio, Fig.4(b)], and the obtained PHA was a copolymer of *β*-hydroxybutyrate and *β*-hydroxyvalerate.

4 DISCUSSION

The concentration and composition of fermentative acids during food wastes anaerobic digestion were affected by pH. More acetic acid could be produced at higher pH, while more propionic, butyric and lactic acids were produced at lower pH. Higher pH in the early stage of food wastes anaerobic digestion stimulated the degradation of food wastes as well as the production of acids. Neutral pH in the late phase of operation, however, caused the consumption of acids instead of their accumulation. It appeared that microorganisms population was shifted from acidogenic one to acid-utilizing one when pH was allowed to evolve itself during the late phase of anaerobic digestion.

For the transfer of acids, dialysis membranes was investigated as the means to combine these two different biological systems. The transferred acids were a mixture of acetic, propionic, butyric and lactic acids with butyric and lactic acids as the major transferred acids. Due to the transfer of propionic acid by dialysis membrane, HV units were synthesized from propionic acid by *R. eutropha*, which could improve properties of the polymer. It is clear that the use of dialysis membrane is an effective way for the transfer of acids from food wastes digestion solution, thus for PHA synthesis. The highest polymer content of 72.6% (ω) PHA was achieved in waste treatment, and comparable with the polymer content obtained in pure glucose fermentation[13].

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