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ORIGINAL PAPER

Ting-Yen Huang · Kow-Jen Duan · Shih-Yow Huang
C. Will Chen

Production of polyhydroxyalkanoates from inexpensive extruded rice bran and starch by *Haloferax mediterranei*

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Abstract Low-cost raw materials can be used to reduce significantly the production cost of polyhydroxyalkanoates (PHA). In this study, extruded rice bran (ERB) and extruded cornstarch (ECS) were used as carbon sources to produce PHA by an archaea, *Haloferax mediterranei*, which cannot use native rice bran or cornstarch as a carbon source. By employing pH-stat control strategy to maintain pH at 6.9–7.1 in a 5-liter jar fermentor using ERB:ECS (1:8 g/g) as the major carbon source, we obtained a cell concentration of 140 g/L, PHA concentration of 77.8 g/L and PHA content of 55.6 wt.% in a repeated fed-batch fermentation. In contrast, when ECS was used as the major carbon source, we obtained 62.6 g/L cell concentration, 24.2 g/L PHA concentration and 38.7 wt.% PHA content. Under a hyper-saline condition and with no nitrogen-limitation restriction, the repeated fed-batch process can be sustained a long time for the mass production of PHA.

Keywords *Haloferax mediterranei* · Polyhydroxyalkanoates · Extruded rice bran · Starch · Repeated fed-batch fermentation

Introduction

Among the several biodegradable polymers under development, PHA has attracted much attention because of similarity of its properties to those of the conventional petrochemical-derived plastics, and its complete biodegradability in various environments. The film-type PHA shows gas-barrier properties comparable to those of poly(vinyl chloride) and poly(ethylene terephthalate); therefore, it can compete with non-degradable polymer used in the packaging industry [9]. PHA can be applied to paper, cardboard, or food trays to form a water-resistant layer as an alternative to polyethylene or aluminum films [2, 16]. PHA of different chemical structures is under investigation for its potential applications in controlled drug release, sutures, bone plates, wound dressing, paramedical disposables, and therapeutic devices [2, 5, 13, 17].

One of the major drawbacks of employing PHA in a wide range of applications is its high production cost. Consequently, much effort has been devoted to reduce the production cost of PHA by improving bacterial strain, efficient fermentation and recovery processes [8]. There are several reasons for using haloarchaea to produce low-cost PHA. In this study, *Haloferax mediterranei* was used to produce PHA under a hyper-saline condition in which very few organisms can survive. The extreme condition of salinity in which this organism grows almost overrides the contamination problem. This greatly reduces the sterility requirements of a production facility and, thus, decreases the investment cost [11, 12]. To study the possibility of long-term culture, *H. mediterranei* was maintained in a chemostat for three months, before confirming that the PHA production yield of final strain was no less than those of the original strain [12]. It is relatively easy to harvest PHA pellet from *H. mediterranei*, compared to other PHA accumulating organisms. As the haloarchaea can be easily lysed in the distilled water and release the PHA pellet to be recovered by low speed centrifugation, the cost of PHA recovery process is greatly reduced [11]. Meanwhile, the authors have developed a high cell density culture system of *H. mediterranei* capable of using cheap starch, rice bran or wheat bran as the major carbon source to produce PHA in a semi-continuous culture process. It had been studied that the cost of carbon source is critical for reducing the production cost of

T.-Y. Huang · K.-J. Duan · C. W. Chen (✉)
Department of Bioengineering, Tatung University,
Taipei, Taiwan
E-mail: will@ttu.edu.tw
Tel.: +886-2-25925252
Fax: +886-2-28349258

S.-Y. Huang
Department of Chemical Engineering,
National Taiwan University, Taipei, Taiwan

PHA [1, 7, 8]. Furthermore, in this study an efficient fermentation system was accomplished based on the signal of pH as well as the conductivity of culture broth, and a pH-stat control strategy set up for medium feeding. The concentration of starch, organic nitrogen, phosphate and salts can be maintained within a proper range that promotes the growth of cell mass and the accumulation of PHA during a repeated fed-batch culture.

Materials and methods

Bacterial strain

H. mediterranei (ATCC 33500) was used throughout this study.

Extrusion process

The extruder used for the starch, rice bran and wheat bran processing was a single-screw extruder (YJ-PS25, Yea Jing Machinery, Taiwan), with a 25 mm inside diameter barrel and four temperature controllers connected to heating/cooling elements on the extruder barrel. The controller maintained the temperature of the extruder within ± 1°C. The set point of temperature in the barrel zones are 55°C for zone 1, 65°C for zone 2, 85°C for zone 3, and 90°C for the extruder die, which has a hole 5 mm in diameter. The die pressure was monitored with a pressure transducer (ISI 0172, Industrial Sensors) to ensure the stability of extrusion process. The extruder is fitted with a screw with length to diameter ratio of 32 and a compression ratio of 2.8. The rotational speed of screw was set at 78 rpm.

1,000 g materials of different weight ratio of rice bran, cornstarch or wheat bran were adjusted to 20% moisture (dry basis) and mixed with α-amylase (BAN 240L, Novo Nordisk Ferment Ltd, Switzerland) at concentration of 5 g enzyme per 100 g of starch. The batch was then fed into the hopper of extruder. The extrudate was collected and dried in an oven at 50°C for 8 h. The dried extrudate was reduced to powders by passing it through a 45-mesh sieve.

Flask culture

Shaking flasks culture for cell growth was under the medium compositions (g/L) as: NaCl 234, $NaHCO_3$ 0.2, NaBr 0.5, $MgSO_4$ 30, $MgCl_2$ 19.5, $CaCl_2$ 1, KCl 5, carbon materials 2, yeast extract (YE) 7.5. Carbon materials include glucose, extruded or native agricultural materials. The cell concentration was estimated by the measurement of optical density $OD_{520\ nm}$. One unit of $OD_{520\ nm}$ is equivalent to 0.42 g/L dried cell weight. The cell growth parameters were estimated by the non-linear regression of the modified Gompertz model [6] as follows:

$$X = A \cdot \exp\left\{-\exp\left[\frac{\mu_m \cdot e}{A} \cdot (\lambda - t) + 1\right]\right\}$$

where X is the cell concentration (g/L) at culture time t (h), A is the maximum cell concentration (g/L), μ_m is the maximum cell growth rate (g/L h), λ is the lag time of cell growth (h), and e is the natural logarithm base.

Repeated fed-batch culture

The repeated fed-batch culture has the same initial medium composition and seed culture as those in the flask culture. The computer control of fermentation system is similar to that described in a previous publication [6]. The measured signals were pH, dissolved oxygen (DO), oxidation-reduction potential (ORP), and weight of fermentor. The culture conditions were set at 37°C with a rotational speed of 800 rpm. Although Rodriguez-Valera and Lillo [14] reported that the best growth temperature for *H. mediterranei* was 45°C, we suggest that a lower temperature 37°C offers two additional advantages: (1) it saves energy cost during long-term continuous culture, and (2) it saves oxygen cost in the high cell density culture since lower temperature promotes a higher saturated oxygen concentration in the broth. Besides, data reported by Lillo and Rodriguez-Valera [12] showed that higher temperature promoted cell growth but decreased PHA content in the cell. The seed culture of 250 mL was transferred to a 5-liter jar fermentor (CMF-5, Firstek, Taiwan) containing 2.5 L of medium. The pH-stat control algorithm was designed to activate medium feeding pump when the pH was increased because of the metabolic activity of cell, and was in the range of 6.9–7.1. If pH was lower than 6.8, 10 N NaOH was added to adjust the pH above 6.8. The stock concentrations of extruded material and YE in the feeding medium were 50 g/L and 85 g/L, respectively. The feeding rate of the pump was set at 0.7 mL/sec. When the volume of broth reached 4.5 L, 2 L of broth would be removed. DO was controlled above 20% saturation. We use conductivity to estimate the total concentration of salt mixture in the fermentation broth. Conductivity was measured by a conductivity meter (SC-12A, Suntex, Taiwan) and was maintained within the range of 3–4 S/m, which represents approximately the total salt mixture concentration of 173–230 g/L. Conductivity was maintained by adding salt mixture with a weight ratio composition of NaCl 234, $NaHCO_3$ 0.2, NaBr 0.5, $MgSO_4$ 30, $MgCl_2$ 19.5, $CaCl_2$ 1 and KCl 5.

Analytical procedures

Starch concentration was quantified by adding 0.4 mL iodine solution (0.2% iodine and 2% potassium iodide)

to a 2 mL diluted supernatant of broth whose $OD_{700\ nm}$ was then measured (modified from Lillo and Rodriguez-Valera [12]). Total Kjeldahl Nitrogen (TKN) refers to the combination of ammonium and organic nitrogen that was converted into ammonium salts by first incubating, for 1.5 h at 300°C, 1 mL supernatant of broth with 4 mL distilled water and 3 mL H_2SO_4, then, followed by incubation with 10 mL 50% H_2O_2 for 15 min at 300°C. The ammonium salts were then analyzed by a modified Nessler method (Method 8075, Hach Co., USA). Phosphate was measured as described by Bae et al. [4]. PHA content was quantified by the chloroform extraction of 2 g lyophilized cell (dried from the lysed cell in the distilled water) in a Soxhlet extractor at 85°C for 6 h.

Results and discussion

Shaking flasks

Lillo and Rodriguez-Valera [12] reported that soluble starch was the best carbon source for *H. mediterranei* growth to produce PHA. But soluble starch is too expensive to be used in a commercial process. Although *H. mediterranei* cannot use native starch, *H. mediterranei* can be used with extruded starch as well as glucose to produce PHA. In this experiment, rice bran and wheat bran were chosen to replace part of starch for economic reason and also for improved PHA productivity.

The comparison of ERB and native rice bran (NRB) was investigated. Without mixing with starch, it is very difficult to extrude the sole rice bran in the single-screw extruder, as we don't have the sole ERB carbon source. The comparison of ERB and NRB was made based on samples mixed with starch (ECS). The tested ERB to ECS weight ratio was 1:8, 1:12 and 1:16. The control groups contained the same ratio of NRB and ECS in medium. Table 1 shows that samples with sole NRB as the carbon source led to very low growth rate, small quantity of cell concentration, and long lag time. The ERB to ECS ratio of 1:8 resulted in the highest growth rate, largest cell concentration, and shortest lag time. Therefore, carbon materials with ERB to ECS ratio of 1:8 were chosen in the repeated fed-batch fermentation.

Table 2 shows the comparison of the cell growth data for EWB (extruded wheat bran) and native wheat bran (NWB). Since we could not obtain sole EWB without mixing with starch during extrusion, the comparison of EWB and NWB was made based on samples mixed with starch (ECS) as shown in Table 2. The tested EWB to ECS ratio was 1:2, 1:3 and 1:4. In contrast to the impropriety of NRB as carbon material (Table 1), Table 2 shows that sole NWB can well be used as a carbon material by *H. mediterranei*, but the use of sole NWB as the carbon source resulted in a longest lag time among all carbon sources in Table 2. The mixture of ECS and NWB or EWB gave rise to similar growth rate and lag time, but EWB enhanced the maximum cell concentration (Table 2). For the economic reason we chose the weight ratio 1:2 of EWB (or NWB) :ECS in the repeated fed-batch fermentation.

Repeated fed-batch fermentation

In this study, the productivity of PHA by *H. mediterranei* was greatly increased by supplying the medium continuously under a pH-stat control strategy to maintain proper concentrations of starch, organic nitrogen and phosphate during fermentation.

Figure 1a–c shows the cell growth, PHA accumulation, controller action and change of broth compositions during the repeated fed-batch culture with the medium composition of ERB and ECS (1:8 w/w) and YE in the feeding stream. The weight ratio of carbon material and YE (C/Y ratio) was kept at 1:1.7 within 118 h of culture. Figure 1a shows that the starch and phosphate concentration were maintained within the range of 10–20 g/L and 0.04–0.08 g/L, respectively. Figure 1b shows that the concentration of organic nitrogen and ammonium were maintained within 0.5–1 g/L and 0.5–1 g/L, respectively. The pH-stat control strategy and the 1:1.7 C/Y ratio prevented the unlimited accumulation or depletion of extruded cornstarch, organic nitrogen and phosphate during fed-batch culture. The pH-stat control

Table 1 Cell growth rate (μ_m), lag time (λ) and the maximum cell concentration (A) on different weight ratio of ERB (extruded rice bran), NRB (native rice bran) and ECS (extruded cornstarch) in the flask culture

Carbon materials	μ_m (g/L h)[a]	λ (h)[a]	A (g/L)[a]
ECS	0.110 ^{A}	55.1 ^{BC}	5.14 ^{BC}
ERB and ECS (1:8)	0.098 ^{A}	47.8 ^{D}	5.22 ^{ABC}
ERB and ECS (1:12)	0.091 ^{A}	51.6 ^{CD}	5.61 ^{A}
ERB and ECS (1:16)	0.086 ^{A}	50.4 ^{CD}	5.56 ^{AB}
NRB	0.006 ^{B}	72.0 ^{A}	0.15 ^{D}
NRB and ECS (1:8)	0.098 ^{A}	58.5 ^{B}	5.12 ^{BC}
NRB and ECS (1:12)	0.109 ^{A}	61.2 ^{B}	4.92 ^{C}
NRB and ECS (1:16)	0.103 ^{A}	59.5 ^{B}	5.05 ^{C}

[a]Values followed by the same letter are not significantly different at P-value of 0.05 according to the Duncan multiple range test

Table 2 Cell growth rate (μ_m), lag time (λ) and the maximum cell concentration (A) on different weight ratio of EWB (extruded wheat bran), NWB (native wheat bran) and ECS (extruded cornstarch) in the flask culture

Carbon materials	μ_m (g/L h)[a]	λ(h)[a]	A (g/L)[a]
EWB and ECS (1:2)	0.094 ^{A}	57.6 ^{BC}	5.32 ^{AB}
EWB and ECS (1:3)	0.102 ^{A}	59.3 ^{B}	5.12 ^{BC}
EWB and ECS (1:4)	0.089 ^{A}	54.5 ^{BC}	5.58 ^{A}
NWB	0.087 ^{A}	67.7 ^{A}	5.47 ^{A}
NWB and ECS (1:2)	0.093 ^{A}	53.2 ^{C}	4.83 ^{C}
NWB and ECS (1:3)	0.095 ^{A}	53.0 ^{C}	4.86 ^{C}
NWB and ECS (1:4)	0.101 ^{A}	53.5 ^{C}	4.90 ^{C}

[a]Values followed by the same letter are not significantly different at P-value of 0.05 according to the Duncan multiple range test

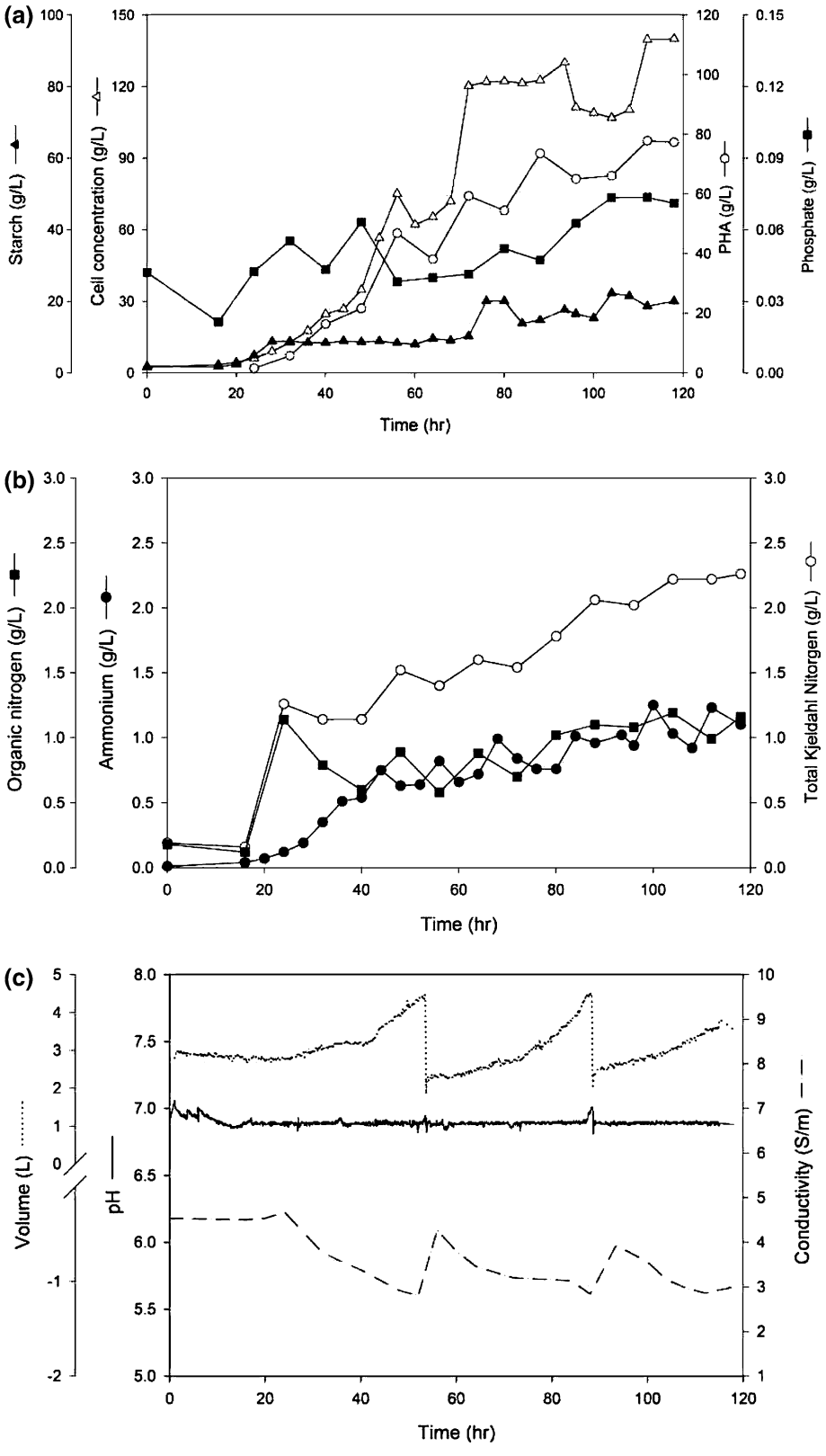

Fig. 1 Repeated fed-batch fermentation of *Haloferax mediterranei* on extruded rice bran and cornstarch (1:8 w/w) under pH-stat control strategy. **a** Time courses of cell, PHA, starch and phosphate concentrations. **b** Time courses of TKN, organic and inorganic nitrogen. **c** Time courses of volume of broth, pH and conductivity

strategy also resulted in a very stable pH curve as shown in Fig. 1c. Figure 1a and b show that the nitrogen source concentrations did not affect the PHA accumulation. In view of the fact that many microorganisms need the nitrogen limitation for the maximum PHA production [17], this will be an advantage for *H. mediterranei* in the repeated fed-batch or continuous culture for the PHA production.

Figure 1a shows that the maximum cell concentration was 140 g/l with 77.8 g/L PHA produced. The synthesis of PHA is triggered by different stimuli including limitation of oxygen, nitrogen, phosphate, sulphur, magnesium or potassium; all these stimuli probably act through the accumulation of NAD(P)H or acetyl coenzyme A, which is necessary for the accumulation of PHA [12, 15, 16]. The limitation of oxygen or nitrogen will hurt the growth of *H. mediterranei* [12]. The limitation of sulphur, magnesium or potassium is not practical for medium containing large quantities of marine salts. In this study, phosphate limitation is considered a probable stimulus. There is no phosphate salt added in the fermentation medium except for phosphate content (6.51 ppm of phosphate) in the YE. Phosphate accumulation in the fermentation broth was determined by the phosphate supply from YE and the microbial consumption rate. Lillo and Rodriguez-Valera [12] reported that 0.00375% wt./vol. (or 0.0375 g/L) of initial KH_2PO_4 concentration stimulated both PHA production and the yield with respect to the carbon source in a low cell density culture (about 10 g/L cell concentration). In this study, during the high cell density culture, phosphate concentration was maintained within 0.04–0.08 g/L as shown in Fig. 1a. We are investigating the phosphate effect on the PHA production by using different agricultural materials except YE.

D'Souza et al. [10] reported that the Haloarchaea requires 200–250 g/L NaCl for optimal growth and lyses when the salt concentration falls below 100 g/L. In this study, for a better automatic control of salts concentration during the fed-batch culture, we use the conductivity signal of growth medium to represent the salts concentration. Therefore, we are investigating the unique role of conductivity in the growth of *H. mediterranei* and PHA production. The conductivity curve is not a stable curve (Fig. 1c) because of the discontinuous addition of salts mixture. The conductivities in Fig. 1c range from 2.8 to 4 S/m which represent the salts mixture (with weight ratio: NaCl 234, $NaHCO_3$ 0.2, NaBr 0.5, $MgSO_4$ 30, $MgCl_2$ 19.5, $CaCl_2$ 1 and KCl 5) of 161 to 230 g/L in the growth medium. A much stable conductivity could be obtained by mixing a proper amount of salts mixture in the feeding materials. The effect of conductivity on the PHA content will be reported in the near future. In Fig. 1a, the decrease of cell concentration from 90 to 110 h is due to the harvest of 2 L broth at 90 h when the broth of culture reached 4.5 L and left the residual broth for the next cycle of fed-batch process. Moreover, we added proper salts mixture after 90 h to increase the conductivity of the broth (Fig. 1c). Similar phenomenon also occurred at 53 h. We could observe the decrease of cell concentration between 53 and 70 h. The removal of 2 L of broth and the addition of salts mixture caused the drastic change of medium composition that affect the cell growth condition. But our past experience indicated that the cell would adapt to the environmental change and re-grow again. We are studying a more stable process, which results in lower cell concentration, by continuously removing broth and adding salts mixture. The results will be reported in our next paper.

Different carbon materials have been used to replace ERB or ECS in the repeated fed-batch culture. Table 3 shows that, compared to ECS, although glucose may promote the growth of cell, it did not promote PHA content. Replacing 1/3 part of ECS by EWB will promote cell concentration significantly compared to non-extruded NWB. Replacing 1/9 part of ECS by ERB will significantly promote cell concentration as well as PHA content. The relevant substrates for the production of PHA include carbon dioxide, fossil resources, renewable resources, waste materials and specialty chemicals [16]. In this study, we use renewable resources (starch) and by-product materials (rice bran and wheat bran) that have economic advantage in the industrial process. In addition, continuous or semi-continuous culture offers many advantages for large-scale production, provided that contamination is avoided and the genetic stability of the strain is guaranteed [12].

Anton et al. [3] mentioned that *H. mediterranei* produced an exocellular polysaccharide (EPS), which was released into the medium, causing an increase in broth viscosity. In the present medium composition, we think it is unlikely that the produced EPS would lead to high viscosity in the culture, which will become an inherent disadvantage in using *H. mediterranei* for PHA production. In that case, we would have a serious cell growth limitation due to the oxygen limitation. We are preparing a paper related to the sulfate-containing EPS and the intracellular polysaccharide (IPS) produced in our system. We have obtained 2.1–5.6 g/L (0.21–0.56% wt./vol.) EPS concentration in our culture broth. According to Anton et al.'s report [3], the

Table 3 Comparison of maximum cell concentration, PHA concentration and PHA content in the repeated fed-batch fermentation on different carbon materials and different weight ratio of ERB (extruded rice bran), EWB (extruded wheat bran), NWB (native wheat bran) and ECS (extruded cornstarch)

Carbon materials	Maximum cell concentration (g/L)	PHA conc. (g/L)	PHA content (wt. %)
ECS	62.6	24.2	38.7
ERB and ECS (1:8)	140.0	77.8	55.6
EWB and ECS (1:2)	131.0	52.7	40.2
NWB and ECS (1:2)	68.4	28.0	40.9
Glucose	85.8	23	27

apparent viscosity of 0.2–0.5% wt./vol. EPS solution was about 6–12 cp, which, from our viewpoint, is not considered highly viscous.

The chemical structure of PHA synthesized by using ECS and yeast extract in the feeding medium was confirmed to be poly(3-hydroxybutyrate-co-3- hydroxyvalerate) (PHBV) copolymer (data have been prepared for publication). We are now investigating the effect of different monomer composition on PHA accumulated in *H. mediterranei* by feeding rice bran, wheat bran or other specialty chemicals. It was reported that PHBV copolymer of a 3-hydroxyvalerate content of about 11 wt.% may have the combined advantage of strength and toughness for wide applications [13].

Conclusion

In this study, inexpensive agriculture materials were extruded and used as a carbon source to produce PHA. A part of ECS was replaced by ERB or EWB. It was found that ERB and EWB not only reduced the cost of material, but also increased the cell concentration and PHA accumulation. In the shaking flask experiment, the authors found that *H. mediterranei* could not be used with NRB for growth, and the cell will grow at a longer lag time when NWB is used. The authors obtained cell concentration of 140 g/L, PHA concentration of 77.8 g/L , and PHA content of 55.6 wt.% by using ERB:ECS (1:8) as the carbon materials in a repeated fed-batch culture. These data represent more than two times that of cell concentration and three times that of PHA concentration produced by using ECS alone. Thus the developed process is potentially viable for commercial, large-scale production of PHA at a continuous mode.

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References

1. Akiyama M, Tsuge T, Doi Y (2003) Environmental life cycle comparison of polyhydroxyalkanoates production from renewable carbon resources by bacterial fermentation. Polym Degrad Stab 80:183–194
2. Anderson AJ, Wynn JP (2001) Microbial polyhydroxyalkanoates, polysaccharides and lipids. In: Ratledge C, Kristiansen B (eds) Basic biotechnology. Cambridge University Press, New York, pp 325–333
3. Anton J, Meseguer I, Rodriguez-Valera F (1988) Production of an extracellular polysaccharide by *Haloferax mediterranei*. Appl Environ Microbiol 54:2381–2386
4. Bae HD, Yanke LJ, Cheng KJ, Selinger LB (1999) A novel staining method for detecting phytase activity. J Microbiol Meth 39:17–22
5. Chen LJ, Wang M (2002) Production and evaluation of biodegradable composites based on PHB-PHV copolymer. Biomaterials 23:2631–2639
6. Chen CW, Lei BC, Yeh KW, Duan KJ (2003) Recombinant sweet potato sporamin production via glucose/pH control in fed-batch culture of *Saccharomyces cerevisiae*. Process Biochem 38(8):1223–1229
7. Choi JI, Lee SY (1997) Process analysis and economic evaluation for poly(3-hydroxybutyrate) production by fermentation. Bioprocess Eng 17:335–342
8. Choi J, Lee SY (1999) Factors affecting the economics of polyhydroxyalkanoate production by bacterial fermentation. Appl Microbiol Biotechnol 51:13–21
9. Choi HJ, Kim J, Jhon MS (1999) Viscoelastic characterization of biodegradable poly(3-hydroxybutyrate-co-3-hydroxyvalerate). Polymer 40:4135–4138
10. D'Souza SE, Altekar W, D'Souza SF (1997) Adaptive response of *Haloferax mediterranei* to low concentration of NaCl (< 20%) in the growth medium. Arch Microbiol 168:68–71
11. Fernandez-Castillo R, Rodriguez-Valera F, Gonzalez-Ramos J, Ruiz-Berraquero F (1986) Accumulation of poly(β-hydroxybutyrate) by halobacteria. Appl Environ Microbiol 51(1):214–216
12. Lillo JG, Rodriguez-Valera F (1990) Effects of culture conditions on poly (β-hydroxybutyric acid) production by *Haloferax mediterranei*. Appl Environ Microbiol 56(8):2517–2521
13. Pachence JM, Kohn J (2000) Biodegradable polymers. In: Lanza RP, Langer R, Vacanti J (eds) Principles of tissue engineering. Academic, New York, p 266
14. Rodriguez-Valera F, Lillo JG (1992) Halobacteria as producers of polyhydroxyalkanoates. FEMS Microbiol Rev 103:181–186
15. Savenkova L, Gercberga Z, Kizhlo Z, Stegantseva E (1999) Effect of phosphate supply and aeration on poly-β-hydroxybutyrate production in *Azotobacter chroococcum*. Process Biochem 34:109–114
16. Steinbuchel A, Fuchtenbusch B (1998) Bacterial and other biological systems for polyester production. Trends Biotechnol 16:419–427
17. Sudesh K, Abe H, Doi Y (2000) Synthesis, structure and properties of polyhydroxyalkanoates: biological polyesters. Prog Polym Sci 25:1503–1555