Iranica Journal of Energy & Environment 1 (2): 132-136, 2010
ISSN 2079-2115
IJEE an Official Peer Reviewed Journal of Babol Noshirvani University of Technology

BUT

Optimization PHAs Production from Dairy Industry Wastewater (Cheese Whey) by *Azohydromonas lata DSMZ 1123*

[1]M. Sharifzadeh Baei, [1]G.D. Najafpour, [1]Z. Lasemi, [2]F. Tabandeh, [3]H. Younesi and [1]H. Issazadeh and [2]M. Khodabandeh

[1]Faculty of Chemical Engineering, Noshirvani University of Technology, Babol, Iran
[2]Biotechnology Department, National Institute of Genetic Engineering and Biotechnology (NIGEB), P.O. Box: 14155-6343, Tehran, Iran
[3]Department of Environmental Science, Faculty of Natural Resources and Marine Sciences, Tarbiat Modarres University, Imam Reza Street, Noor, Iran P.O. Box: 46414-356

Abstract: In the present research, whey was used as useful substrate which retained from permeates of dairy industry. The obtained whey was hydrolyzed to cleave its main carbon source, lactose to glucose and galactose.The hydrolyzed products were chosen as carbon sources for the production of poly-3-hydroxybutyric acid (PHB) by *Azohydromonas lata DSMZ 1123*. The biosynthesis of PHA copolyesters containing 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) units from hydrolyzed whey permeate and valerate were investigated. The application of hydrolyzed whey permeate turned out to be advantageous compared to utilization of pure sugars. Therefore, fermentation under controlled conditions (agitation rate of 250 rpm, 18 h incubated inoculum, 72 h fermentation time and temperature set at 30°C) was performed. As a result, maximum amount biopolymer obtained was1.66 g/L. The biopolymer consisted 1.21 and 0.45 g/L of P(3HB) and P(3HV), respectively.

Key words: *Azohydromonas lata* · Cheese whey · Lactose · 3-hydroxybutyrate (3HB) · 3-hydroxyvalerate (3HV) · Polyhydroxyalkanoate

INTRODUCTION

Polyhydroxyalkanoates (PHAs) are bacterial polyesters that have received great interests in recent years. PHAs have been considered to be good substitutes for petroleum-derived synthetic plastics showing similar properties as synthetic polymers and furthermore they degrade after disposal [1, 2]. PHAs are synthesized by several bacterial species and accumulated as energy and/or carbon storage materials, usually when supplementary nutritional factors such as nitrogen, phosphorus, potassium, oxygen, sulfur or magnesium are limited in the presence of an excess carbon source [3, 4]. PHAs are divided into three categories depending on the number of carbon atoms in their monomer units; short chain-length (SCL), medium-chain-length (MCL) and long chain-length (LCL). The three categories, SCL, MCL and LCL are composed of hydroxyl acids with 3-5, 6-14 and more than 14 carbon atoms, respectively [2, 5]. The flexibility of the enzymes which are responsible for PHA production, to incorporate monomers of various length lead to a family of biopolyesters with many structural possibilities and a wide-range field of applications [6]. Whey is the major by-product of cheese production processes, representing 80-90 % of the volume of milk transformed [7, 8]. Only half of the whey produced is employed for its transformation into useful products such as food ingredients and human and animal feed, whereas the rest is encountered as a pollutant due to its high biological oxygen demand. Whey has been used for PHB production using *Azohydromonas lata DSMZ 1123*, either in batch cultures [9, 10] or in laboratory scale fermenters [8, 10-13]. The possibility of direct conversion of whey lactose to PHA, using the wild type stains of *Hydrogenophaga pseudoflava* DSM 1034 and *Sinorhibium melitoti* 41 have been investigated [14].

Corresponding Author: Dr. G.D. Najafpour, Faculty of Chemical Engineering, Noshirvani University of Technology, Babol, Iran
E-mail: najafpour@nit.ac.ir. Fax: +98-111-3210975

Recently, the ability of the *halophilic archaeon*, *Haloferax mediterranei* as well as *Eubacterial*, *Pseudomonas hydrogenovora* and *Hydrogenophaga pseudoflava* to utilize whey for PHA production was studied [15, 16].

On the other hand, whey is an attractive and potential raw material for PHA production but the inability of most PHA bacteria to utilize lactose, has been restricted to its use as a carbon source [17].

The purpose of this research work was to synthesis PHAs by *A. lata* DSMZ 1123 with sufficient source of carbohydrates, in a medium mainly consisted of whey (lactose) as sole source of energy. PHAs were accumulated insides the cell bodies of *A. lata* under optimal media and growth conditions.

MATERIALS AND METHODS

Maintenance of Stock Culture: The bacterial strain used in the present study was *Azohydromonas lata* DSMZ 1123. It was maintained on Luria Agar slants at 4°C and sub cultured every 15 days to maintain its viability.

Media: Whey was obtained from Gela Industry (Amol, Ian). Whole whey solution was uniformly acidified by acid solution (HCl, 5 N) at acidic pH (less than 4) to remove excessive proteins [17]. The solution was autoclaved at 15psig, 121°C for 15 min, then cooled down to room temperature, centrifuged at 11,000 x g in sterilized tubes for 15 min to remove aggregated solids. The supernatant (whey supernatant), was refrigerated for 12 hours and it was used after adjusting pH to 7 by the concentrated NaOH solution (5M), as the major constituent of media for the growth of *A. lata* in all experiments.

Optimization Tests: The seed culture of *A. lata* was incubated at 30°C for 24 h. The resultant cultures were transferred to a 1000ml flask containing 200 ml seed culture medium. The flasks were incubated at 30°C and agitated at 200 rpm for 10, 15, 20 and 30 h to investigate the effect of the seed age. The inoculum size was set at about 10 v%. In order to investigate the effect of temperature and agitation rates on dry cell weight (DCW) and PHB accumulation, sever sets of experiments were conducted at following experimental conditions: temperature (25, 27, 30 and 33°C), agitation rate (150, 200, 250 and 300 rpm).

Dry Cell Weight: The cell concentration of the cultured media was determined by the cell optical density at 620 nm with the aid of a spectrophotometer (UNICO2100, USA) after suitable dilution with distilled water. The cell dry weight was also measured based on standard calibration curve of absorbance as a function of cell dry weight for the pure culture of *A. lata*.

Biopolymer Extraction: Extraction of biopolymer was conducted according to the method developed by Braunegg [7]. For polyester quantification, a 5 mL of culture broth was centrifuged at 3600 rpm for 20 min. A solution of 2 mL of chloroform and 2 mL of acidified methanol (3% sulfuric acid) were added to the cell pellet in vial with Teflon screw cap and heated at 100°C for 3.5h.

Determination of Total Carbohydrate Concentration: Supernatant was used for residual nutrient analysis including total sugar. The method is based on total reducing sugar by reagent of dinitrosalicilic acid (DNS) method [18]. Standard method was developed using the reagent for colorimetric method to detect orange color using spectrophotometer at wavelength of 580 nm.

Biopolymer Analysis: Gas chromatography (GC) was performed by using a gas chromatograph (Philips PU4400, US), equipped with flame ionization detector (FID) and data acquisition system with computer software (Clarity 4.2, Data Apex, Czech Republic), used for the methyl-3-hyroxybutyrate (3HB) analysis. The GC column, capillary column (BP20 SGE, Australia) with 0.33 mm internal diameter, 25 m length was used. The column temperature was initially maintained at 80 °C for 4 min, followed by a temperature program at a rate of 8 °C/min till it reached 160°C, maintained for 3 min and then at a rate of 30°C/min increased to 200 °C. The detector and injector temperatures were 280 and 250 °C, respectively. The gases used were helium as a carrier gas with a flow rate of 1.5 mL/min, hydrogen 30 mL/min and air 300 mL/min. The injection volume size was 1 µL of the prepared samples.

RESULTS AND DISCUSSION

Batch experiments were conducted using whey as major source of maintaining energy for cell metabolisms. More lactose consumptions may result in high PHBs production. Since the synthesis of PHAs was intracellular product, high cell concentration may lead to more biopolymer. Based on literature review, optimal media composition with suitable carbon source should be in favor of PHAs production with maximum cell growth of *A. lata*. A few process variables were investigated for maximum productivity of PHAs. The media composition was investigated in previous experiment [19].

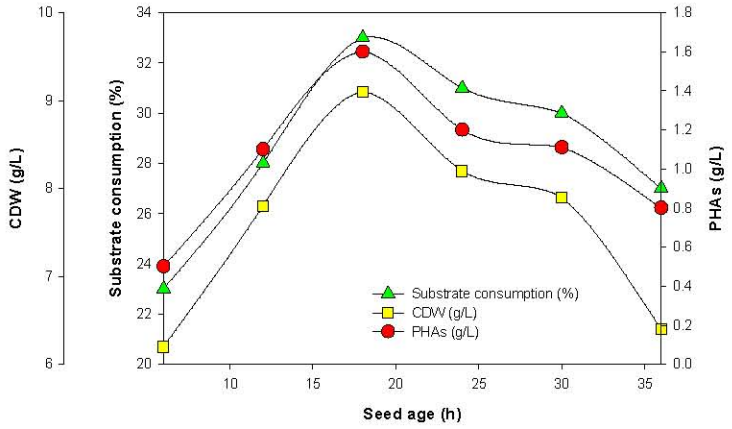

Fig. 1: Effect of seed age on cell growth and PHAs production (operating conditions: T = 30°C, agitation rate = 250 rpm).

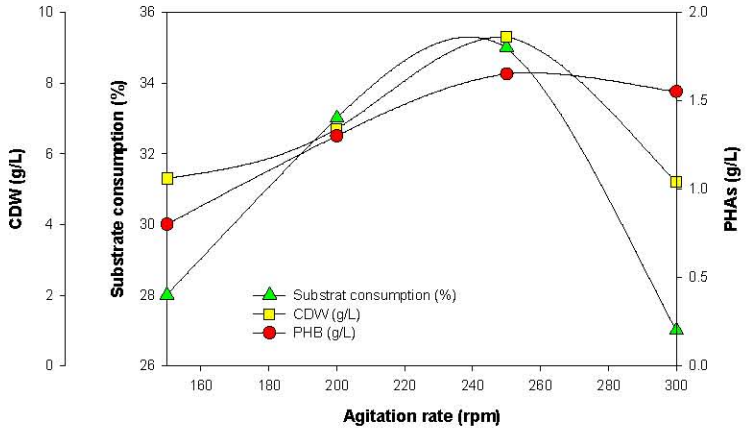

Fig. 2: Effect of seed agitation rate on cell growth and PHAs production (operating conditions: T = 30°C, seed age = 18 h).

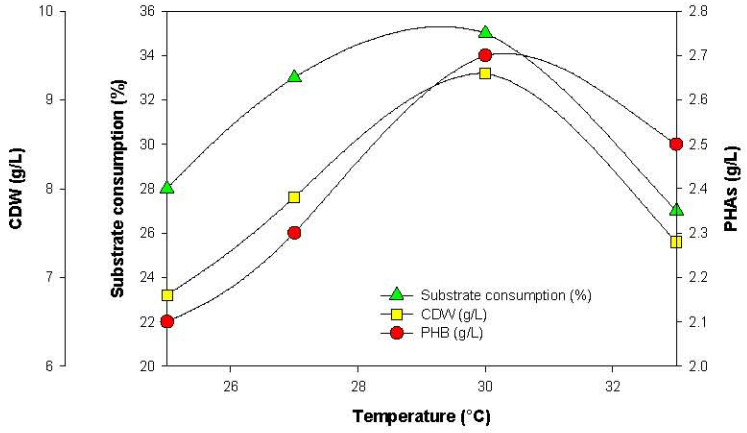

Fig. 3: Effect of temperature on cell growth and PHB production (operating conditions seed age = 18h, agitation rate = 250 rpm).

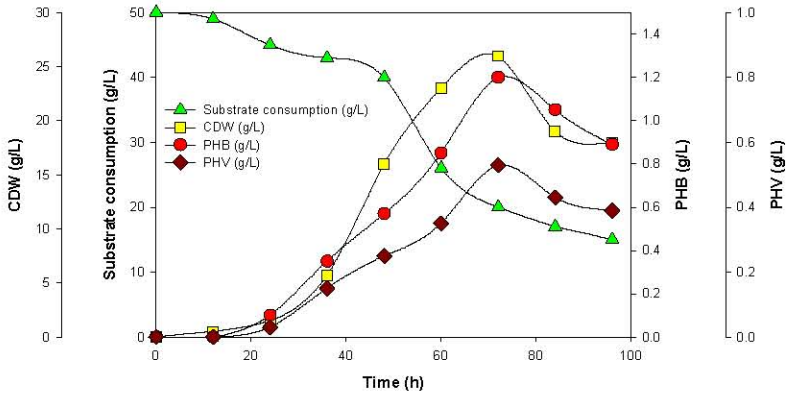

Fig. 4: Concentration profile for cell growth, substrate utilization and biopolymers (PHB and PHV) production at (operating condition: T = 30°C, agitation rate = 250rpm, seed age = 18 h).

The lactose as carbon source was supplied by the treated whey as low costs and abundant source for ensuring sufficient cell of *A. lata* grow in the selected media. The effect of seed age on cell growth and PHAs production is illustrated in Figure 1. It was observed that the cell growth and PHAs accumulation are gradually increased as the seed age reached to 18 h and then there was slightly decrease. Since seed age of *A. lata* was at the mid-exponential phase at 15 h, that inoculums contained alive and active bacteria. Under this condition, DCW was 9.2 and 1.66 g/L of CDW and biopolymers were obtained.

The effect of agitation rate on growth and PHB production was investigated. Figure 2 shows at agitation rate of 250 rpm maximum cell growth and PHB production were achieved. Agitation rate of higher than 250 rpm cell lysis occurred and productivity of PHB was declined.

Figure 3 depicts the effect of media temperature on cell growth and PHB production. Media temperature effect on cell growth and PHB production was monitored while keeping other parameters such seed age at 18 h and agitation rate of 250 rpm. The results indicated that at 30°C, maximum DCW and biopolymer (PHB and PHV) were obtained. The concentration of DCW and biopolymer were 9.35 and 1.7g/L, respectively.

The concentration profile for cell growth, substrate utilization and biopolymers (PHB and PHV) production at defined operating conditions: (T = 30 °C, agitation rate = 250 rpm, seed age = 18 h) are shown in Figure 4. The data shows that about 70% of lactose was converted to biopolymers and biomass.

CONCLUSION

It was concluded that the pretreated whey was a suitable substrate for the production of biopolymers (PHBs and PHVs). The process variables were analyzed for maximum productivity of the biopolymers. The optimal conditions for PHBs and PHVs productions were at 30°C, agitation rate of 250 rpm and the seed age of 18 h. Maximum production of biopolymer and CDW were 1.66 and 9.3 g/L, respectively.

ACKNOWLEDGEMENTS

The authors are grateful to Department of Environmental Science, lab of Natural Resources and Marine Sciences, Tarbiat Modarres University for their great assistant on preliminary sample analysis.

REFERENCES

1. Byrom, D., 1987. Polymer synthesis by micro-organisms: technology and economics. Trends Biotechnol., 5: 246-250.
2. Anderson, A.J. and E.A. Dawes, 1990. Occurrence, metabolism, metabolic role and industrial uses of bacterial polyhydroxyalkanoates. Microbiol. Rev., 54: 450-472.
3. Du, G., Y. Si and J. Yu, 2001. Inhibitory effect of medium-chain-length fatty acid on synthesis of polyhydroxyalkanoates from volatile fatty acid by *Ralstonia eutrophus*. Biotechnol. Lett., 23: 1613-1617.
4. Du, G. and J. Yu, 2002. Metabolic analysis on fatty acid utilization by *Pseudomonas oleovorans*: mcl-poly-(3-hydroxyalkanoates) synthesis versus oxidation. Process Biochem., 38: 325-332.
5. Steinbüchel, A., 1992. Biodegradable plastics. Curr. Opin. Biotechnol., 3: 291-297.
6. Choi, J. And S.Y. Lee, 1997. Process analysis and economic evaluation for poly(3-hydroxybutyrate) production by fermentation. Bioprocess Eng., 17: 335-342.

7. Pantazaki, A.A., C.P. Papaneophytou, A.G. Pritsa, M. Liakopoulou-Kyriakides and D.A. Kyriakidis, 2009. Production of polyhydroxyalkanoates from whey by *Thermus thermophilus* HB8. Process Biochem., 44: 847-853.
8. Wong, H.H. and S.Y. Lee, 1998. Poly-(3-hydroxybutyrate) production from whey by high density cultivation of recombinant *Escherichia coli*. Appl. Microbiol. Biotechnol., 50: 30-33.
9. Fidler, S. And D. Dennis, 1992. Polyhydroxyalkanoate production in recombinant *Escherichia coli*. FEMS Microbiol. Rev., 103: 231-236.
10. Lee, S.Y., A.P.J. Middelberg and Y.K. Lee, 1997. Poly(3-hydroxybutyrate) production from whey using recombinant *Escherichia coli*. Biotechnol. Lett., 19: 1033-1035.
11. Ahn, W.S., S.J. Park and S.Y. Lee, 2000. Production of poly (3-hydroxybutyrate) by fed-batch culture of recombinant *Escherichia coli* using highly concentrated whey solution. Appl. Environ. Microbiol., 66: 3624-3627.
12. Ahn, W.S., S.J. Park and S.Y. Lee, 2001. Production of poly(3-hydroxybutyrate) from whey by cell recycle fed-batch culture of recombinant *Escherichia coli*. Biotechnol. Lett., 23: 235-240.
13. Park, S.J., J.P. Park and S.Y. Lee, 2002. Production of poly(3-hydroxybutyrate) from whey by fed-batch culture of recombinant *Escherichia coli* in a pilot-scale fermenter. Biotechnol. Lett., 24: 185-189.
14. Povolo, S. And S. Casella, 2003. Bacterial production of PHA from lactose and cheese whey permeate. Macromol, Symp., 197: 1-9.
15. Koller, M., P. Hesse, R. Bona, C. Kustschera, A. Atliæ and G. Braunegg, 2007. Potential of various archae-and eubacterial strains as industrial polyhydroxyalkanoate producers from whey. Macromol. Biosci., 7: 218-226.
16. Koller, M., R. Bona, E. Chiellini, F. Fernades Grillo, P. Horvat, C. Kustschera, P. Hesse and G. Braunegg, 2008. Polyhydroxyalkanoates production from whey by *Pseudomonas hydrogenovora*. Bioresour. Technol., 99: 4854-4863.
17. Yellore, V. And A. Desai, 1998. Production of poly-3-hydroxybutyrate from lactose and whey by *Methylobacterium* sp. ZP24. Lett. Appl. Microbiol., 26: 391-394.
18. Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 31: 426-8.
19. Daneshi, A., H. Younesi, M. Ghasempouri and M. Sharifzedeh, 2008. Production of biodegradable biopolymer, poly-3-hydroxybutirate, from corn syrup by *Ralstonia eutropha*: Effect of nitrogen source. J. Biotechnol., 136: S402-S459.