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Production of Polyhydroxyalkanoates by *Azotobacter vinelandii* UWD Using Swine Wastewater: Effect of Supplementing Glucose, Yeast Extract, and Inorganic Salts

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Abstract This study examined the effect of adding glucose, yeast extract, and inorganic salts to swine wastewater (SWW) in a batch culture on the production of a biodegradable plastic, polyhydroxyalkanoate (PHA). A bacterial strain, *Azotobacter vinelandii* UWD, was used to produce PHA without limiting the non-carbon nutrients. The addition of glucose (30 g/L) to the SWW medium increased the level of cell growth (4.4~7.0 times) and PHA production (3.8~8.5 times) depending upon the dilution of SWW. A 50% dilution of SWW was found to be optimal considering the dry cell weight (9.40 g/L), PHA content (58 wt%), and hydroxyvalerate (HV) mol fraction in the PHA (4.3 mol%). A 75% SWW medium was more advantageous for producing PHA with a higher HV fraction (7.1 mol%) at the expense of losing 22% of PHA production. The undiluted SWW medium produced less than one third of the PHA compared with the 50% SWW medium, but the HV fraction was the highest (10.8 mol%). Regarding the effect of the glucose concentration, at 20 g/L glucose, the dry cell weight and level of PHA production increased to 9.34 g/L (0.63 g PHA/g dry cell weight) and 5.90 g/L, respectively. At 50 g/L glucose, there was no significant increase in PHA production. For the glucose-supplemented (30 g/L) 50% SWW medium, the addition of a nitrogen source (1 g/L of yeast extract) did not increase the level of cell growth or PHA production because the C:N ratio (23:1) was already close to the optimal value (22:1). Better aeration increased the productivity of PHA. External nitrogen supplements (1 g/L of yeast extract) and other essential mineral salts was not necessary for bacterial growth because they were contained in the SWW. These results suggest that SWW is an excellent feedstock for producing larger amounts of the value-added material, PHA, if it is combined with carbohydrate-rich organic waste. © KSBB

Keywords: polyhydroxyalkanoates, swine wastewater, Azotobacter vinelandii*, glucose*

INTRODUCTION

Plastics made by the petrochemical industry are essentially nondegradable substances in landfill and cause serious air pollution problem if incinerated. Biodegradable plastics, such as polyhydroxyalkanoates (PHA), polysaccharides, polylactides, and aliphatic polyesters, have been developed as an alternative to petrochemical plastics [1-5]. Among them, PHA has attracted increasing attention because their properties are similar to conventional plastics [1-5].

PHA is a microbial reserve polyester that bacteria accumulate intracellularly as both a carbon and energy reserve material [1-9]. Among the various PHAs, poly-3-hydroxybutyrate (PHB) and poly-3-hydroxybutyrate-co-3-hydroxyvalerate, P (3HB-co-3HV), have attracted most attention [1-5]. Several studies have examined PHA production using bacteria such as *Alcaligenes eutrophus* [9-11], *Ralstonia eutropha* [6-12], *Azotobacter vinelandii* [13-16], *Methylobacterium* sp. [17], *Pseudomonas* sp. [18,19], and recombinant *E. coli* [3,5,20].

Despite the environmental advantages and extensive re-

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search activity, PHA is not used widely due to its relatively high production cost [21]. The production costs can possibly be lowered if an inexpensive fermentation feedstock can be found. There are many reports on the production of PHA using carbon rich feedstock, such as molasses, malt extract and corn syrup [14,22], and organic wastes [11,18,23-26]. Swine wastewater (SWW) is a feedstock with quite a different composition that is rich in nitrogen, phosphorus, and mineral salts. SWW, which normally causes significant environmental problems, will provide significant environmental and economic advantages if it is used in the production of PHA. However, in order to utilize SWW as a feedstock for PHA production it is important to determine how to reduce the concentration of the non-carbon nutrients (nitrogen, phosphorous, *etc.*) present in SWW because most PHA-producing bacteria (e.g., *A. eutrophus*) require limited supplies of non-carbon nutrients for PHA accumulation. This problem can be solved by using *A. vinelandii* UWD because this strain produces PHA without the need for nutrient limitation [14,22]. A previous study reported that *A. vinelandii* UWD can produce PHA using an alkanoate mixture or SWW as a feedstock. However, cell growth and PHA production is limited to a very low level [15,16]. This study examined the effects of the glucose concentration, aeration and the addition of yeast extract and mineral salts on the production of PHA by this bacterium in order to optimize the production of PHA from SWW.

MATERIALS AND METHODS

Swine Wastewater (SWW)

SWW was obtained from the Southern Experiment Station of the University of Minnesota and stored at −20°C. The SWW was centrifuged, and the supernatant was autoclaved at 120°C for 30 min prior to use as a medium for the production of PHA. The SWW contained alkanoates, such as acetate, propionate, butyrate, *iso*-butyrate, valerate, *iso*-valerate, and hexanoate [16]. Nitrogen and phosphate mineral salts, such as K, Ca, Mg, and Na, were also found in SWW [16]. The C:P, C:N, and N:P ratios in the SWW were approximately 81:1, 11:1, and 8:1, respectively [14].

Bacterial Strain and Maintenance Medium

A mutant strain UWD of *A. vinelandii* (ATCC 53799) was cultured and maintained in a glucose medium containing 30 g/L glucose and mineral salts of the following composition (g/L): KH_2PO_4 0.3, $MgSO_4$ $7H_2O$ 0.3, $CaSO_4{\cdot}2H_2O$ 0.015, CH_3COONH_4 1.1562, ferric citrate 0.01029, $Fe_2SO_4{\cdot}7H_2O$ 0.0075, $Na_2MoO_4{\cdot}2H_2O$ 0.00036, and yeast extract 1. The pH of the glucose medium was adjusted to 7.3 by adding a 0.5 N NaOH solution.

Cell Growth and PHA Production in SWW Medium

The inoculum was 4% (v/v) and pregrown for 24 h in a glucose-alkanoates medium. The glucose-alkanoate medium was a modified glucose medium in which CH_3COONH_4 was replaced with 1.605 g/L of NH_4Cl, and three different alkanoates (3.8 g/L of acetate, 1.0 g/L of propionate, and 1.0 g/L of butyrate) were supplemented.

The level of cell growth and PHA production were examined using undiluted SWW and SWW diluted with distilled water (DW). The SWW from four different dilutions was used: 100% SWW (undiluted SWW), 75% SWW, 50% SWW, and 25% SWW. The following inorganic salts were added to the undiluted and diluted SWW: (in g/L) KH_2PO_4, 0.3; $MgSO_4{\cdot}7H_2O$, 0.3; $CaSO_4{\cdot}2H_4O$, 0.015; NH_4Cl, 1.605; $Fe_2SO_4{\cdot}7H_2O$, 0.0075; $Na_2MoO_4{\cdot}2H_2O$, 0.00036. The salt-supplemented SWW is referred to in this paper as the SWW medium. Fermentation was carried out in duplicate at 30°C for 48 h in 250 mL flasks using a working volume of 100 mL. The agitation speed was 200 rpm.

The effect of adding an extra carbon source on PHA production was examined by supplementing the SWW at four different dilutions with 30 g/L of glucose. The effect of the glucose concentration on the level of PHA production was also studied by adding 10 to 50 g/L glucose to a 50% SWW medium. The effect of aeration on the level of PHA production was examined by comparing the results of two different culture volumes (50 and 100 mL working volume) using a 50% SWW medium supplemented with 30 g/L of glucose. The effect of yeast extract supplementation (1 g/L) on the production of PHA was also examined using 50% SWW medium supplemented with 30 g/L of glucose. As a comparison, the production of PHA was also examined in a 30 g/L glucose medium without SWW. The effect of inorganic salts on the level of PHA production was examined by comparing the results with and without mineral salt supplementation to a 50% SWW medium or undiluted SWW medium, both containing 30 g/L of glucose.

Analytical Procedure

Ten mL of the culture broth was withdrawn periodically from each shake flask for analysis. The cells were obtained by centrifugation at 10,000 rpm for 10 min and used to determine the cell dry mass and PHA content. The supernatant obtained by centrifugation was analyzed for alkanoates and glucose. After washing with distilled water, the harvested cells were dried at 110°C for 24 h to determine the dry cell mass. PHA was extracted from the dry cells by acid-propanolysis, and the PHA concentration was measured by gas chromatography (GC, HP 5890, USA) [16]. The residual non-PHA cell mass was calculated by subtracting the PHA dry mass from the total dry cell mass [16].

To analyze the alkanoates, 0.5 mL of the supernatant prepared by centrifugation was placed into a GC vial containing 0.5 mL of 0.5 N HCl, and the concentration of alkanoates was measured by GC [16]. The glucose concentration was measured using a glucose analyzer (YSI model 27, USA).

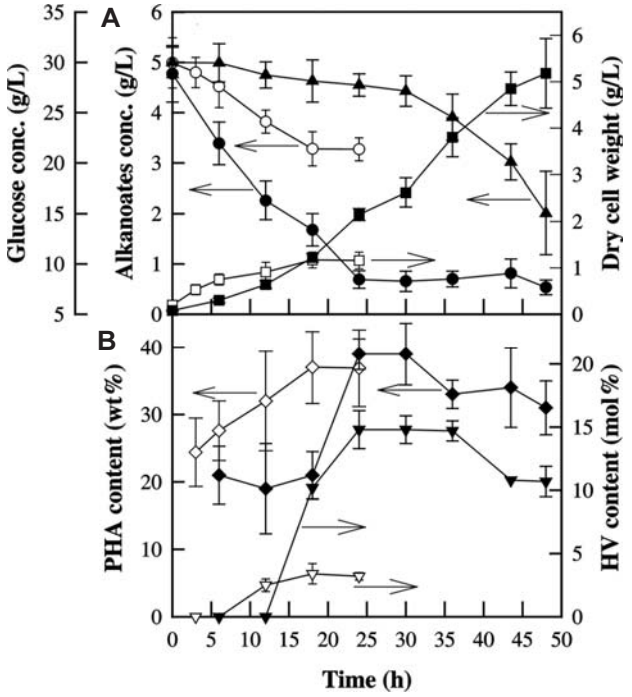

Fig. 1. Cell growth and production of PHA by *Azotobacter vinelandii* UWD in 100% SWW medium. Open symbol, no addition of glucose; closed symbol, addition of 30 g/L glucose. Symbols: ○●, alkanoates concentration; ▲, glucose; □■, dry cell weight; ◇◆, PHA content; ▽▼, HV content.

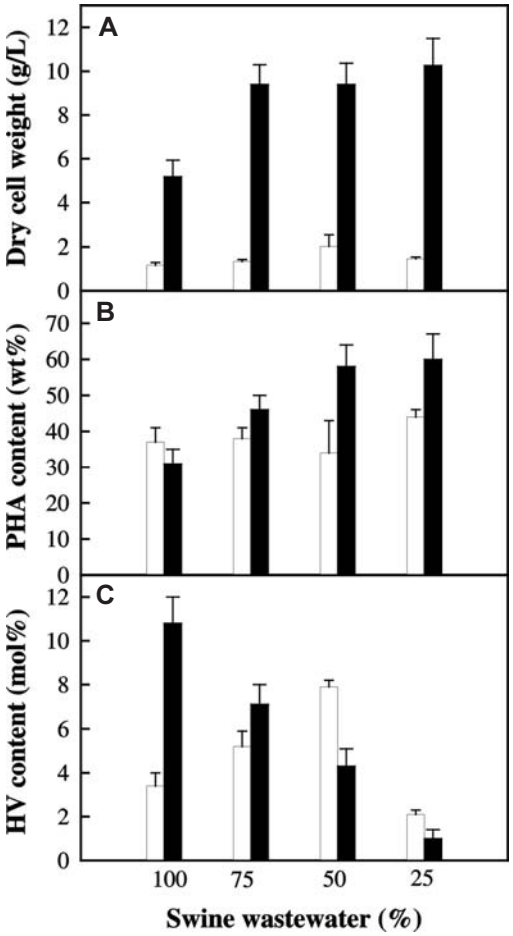

Fig. 2. Effect of glucose addition on the level of cell growth and PHA production in 100, 75, 50, and 25% SWW media. □, no addition of glucose (the results were cited from Ref. 18); ■, addition of 30 g/L glucose.

RESULTS AND DISCUSSION

Effect of Glucose and SWW Dilution

Adding glucose to the undiluted SWW medium (100% SWW medium) increased the growth of *A. vinelandii* UWD and the level of PHA production. After adding 30 g/L glucose to the undiluted SWW medium, the dry cell mass, PHA mass, and PHA content reached 5.18 g/L, 1.62 g/L, and 31 wt%, respectively, after 48 h incubation (Fig. 1). The level of cell growth and PHA production were 4.4 and 3.8 times higher, respectively, than that reported in previous work without the addition of glucose to the undiluted SWW medium [16]. In previous work, cell growth was limited to 1.17 g/L with 0.43 g/L PHA [18]. With glucose supplementation (30 g/L), the HV fraction in PHA (10.7 mol%) was also 3.1 times higher than in previous studies (3.4 mol%) [16]. HV repeating units in the polymer chains reduce the hardness, crystallinity and melting point of the polymer, thereby increasing its impact strength and processability [8].

In addition to the increase in PHA production, the cell growth pattern changed significantly when glucose was added to the undiluted SWW medium. With 30 g/L glucose supplementation, *A. vinelandii* UWD exhibited a diauxic growth pattern by switching between carbon sources. First this bacteria utilized alkanoates originally present in the SWW, and then consumed the supplemented glucose when the alkanoates were depleted (Fig. 1). When alkanoates were used, the PHA content (wt%) in the cell was relatively constant at approximately 20 wt% and cell growth was rather slow. However, the PHA content almost doubled to 39 wt% and the cell growth rate increased when the cells began to consume glucose preferentially. The HV fraction in PHA reached a maximum of 14.8 mol% after 24 h fermentation when using alkanoates but decreased to 10.7 mol% at the end of the glucose consumption phase (Fig. 1).

The dilution of SWW influenced the effect of glucose addition on cell growth and PHA production. For all the three diluted SWW media (75%, 50%, and 25% SWW medium), 30 g/L glucose addition increased the level of cell growth and PHA production compared with the undiluted SWW medium. For the diluted SWW medium, the level of cell growth and PHA production increased 4.7~7.0 and 7.9~8.5 times, respectively, depending on the dilution ratio (Fig. 2). This remarkable increase in cell growth and PHA production with glucose supplementation suggests that the production of

PHA from SWW might be cost-effective if the SWW feedstock can contain or be supplemented with other carbon-rich agricultural or food processing waste [27,28].

Among the three diluted media, 50% SWW medium appeared the best for cell growth (9.4 g/L), PHA content (58 wt%), and HV mole fraction in the PHA (4.3 mol%). The level of cell growth (10.3 g/L) and PHA content (60 wt%) were slightly better in the 25% SWW medium than in 50% SWW medium. However, the HV content in the PHA was marginal (1.0 mol%) (Fig. 2). Therefore, 25% SWW medium is not recommended in cases where the material properties of PHA are considered important. It was reported that the physical properties of PHA, such as impact strength and flexibility, improve with increasing HV fraction [1-3]. In the case of the 75% SWW medium, the PHA content (46 wt%) was lower than that obtained in the 50% SWW medium (58 wt%) with the same level of cell growth (9.4 g/L). On the other hand, the 75% SWW medium was more advantageous for producing a PHA polymer with a higher HV fraction (7.1 mol% vs. 4.3 mol% for the 50% SWW medium) (Fig. 2).

A previous study using SWW medium without glucose supplementation also showed that the level of PHA production was best in the 50% SWW medium, and further dilutions decreased the cell mass and PHA production [16]. However, those previous results are not practical because of the limited cell growth (dry cell weight was 2.02 g/L with 0.69 g/L of PHA).

This study shows that glucose supplementation (30 g/L) is the least beneficial for increasing the level of cell growth and PHA production (4.4 and 3.8 times, respectively) when the SWW was undiluted (Fig. 2). This appears to be because the high concentration of alkanoates in undiluted SWW inhibits cell growth. Previous work showed that the dilution of SWW decreased the alkanoate concentration below the inhibitory level [16]. Similar results were also reported by Page *et al.* [13]. On the other hand, the HV fraction in the produced copolymer, P(HB-co-HV), was best (10.8 mol%) in the undiluted SWW medium, as evidenced by the decrease in HV fraction from 7.1, to 1.0 mol% at 75% to 25% dilution, respectively (Fig. 2). This suggests that undiluted SWW medium is the best choice when the aim is to produce PHA polymers with higher impact strength and flexibility but at the expense of lower cell growth and PHA production.

Not only did the dilution of SWW modify the effect of glucose addition on cell growth and PHA production, it also affected the yield of PHA per residual non-PHA biomass ($Y_{PHA/RM}$, g/g). The residual non-PHA biomass is the biomass not including PHA. The values of $Y_{PHA/RM}$ (g/g) for the undiluted, 75%, 50%, and 25% SWW medium were 0.45, 0.84, 1.40, and 1.48, respectively. The $Y_{PHA/RM}$ (g/g) value of the 25% SWW medium (1.48) was 3.3 times higher than that of the undiluted SWW medium (0.45), whereas the dry cell weight (10.26 g/L) of the 25% SWW medium was only double that of the undiluted SWW medium (5.18 g/L). This suggests that PHA production is more preferential than non-PHA biomass formation when the *A. vinelandii* UWD cells are grown in a more diluted SWW medium. It was presumed that the carbon flux in the PHA biosynthesis pathway is greater than the carbon flow for the formation of residual non-PHA biomass when the cells encounter a relatively high glucose to alkanoate ratio.

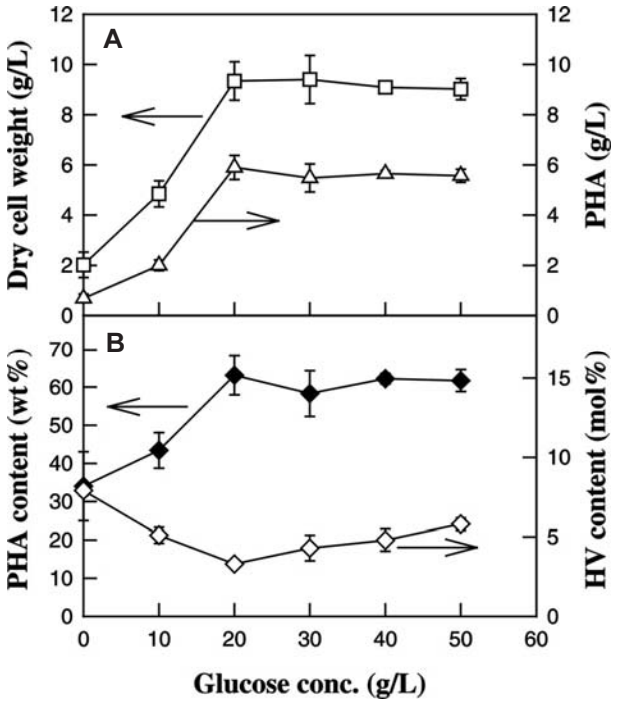

Fig. 3. Effect of glucose concentration on the level of cell growth and PHA by *Azotobacter vinelandii* UWD in 50% SWW medium. Symbols: (A) □, dry cell mass; △, PHA mass. (B) ◆, PHA content (wt%); ◇, HV mol%.

Effect of Glucose Concentration

The effect of the glucose concentration on cell growth and PHA production was examined by supplementing the 50% SWW medium with up to 50 g/L of glucose (Fig. 3). The dry cell weight, PHA production, and PHA content increased to 9.34 g/L, 5.90 g/L, and 63 mol%, respectively, when the glucose concentration was increased to 20 g/L. Further increases in glucose concentrations up to 50 g/L did not produce a noticeable increase in the dry cell weight and PHA production (g/L).

Effect of Aeration on PHA Production in 50% SWW Medium

Aeration affected cell growth and PHA production in a 250 mL flask culture using 50% SWW medium. The aeration rate was varied by using a different culture volume (50 and 100 mL) in the same sized (250 mL) flask. When 50 mL of the culture volume was used, the dry cell mass, PHA production and rate of PHA production were 12.8 g/L, 7.39 g/L and 0.11 $g \cdot L^{-1} \cdot h^{-1}$, respectively (Table 1). The level of cell growth and PHA production and PHA productivity were 1.4,

Table 1. Effect of aeration (medium volume) on the production of PHA in 50% SWW medium supplemented with 30 g/L of glucose

Initial volume (mL)	DCW (g/L)	PHA (g/L)	% PHA (dry wt)	HV (mol%)	$Y_{PHA/RM}$[a]	PHA (g L^{-1} h^{-1})
100	9.40 ± 0.96	5.48 ± 0.56	58 ± 6	4.3 ± 0.8	1.40 ± 0.14	0.11 ± 0.01
50	12.84 ± 1.80	7.39 ± 1.04	58 ± 8	4.1 ± 0.4	1.36 ± 0.19	0.15 ± 0.02

[a]Yield in grams of PHA per gram of residual non-PHA biomass.

Table 2. Effect of the yeast extract on the production of PHA

SWW (%)	Glucose (g/L)	Yeast extract (g/L)	DCW (g/L)	PHA (g/L)	% PHA (dry wt)	HV (mol%)	$Y_{PHA/RM}$[a]	PHA (g L^{-1} h^{-1})
50	30	1	9.40 ± 0.96	5.48 ± 0.56	58 ± 6	4.3 ± 0.8	1.40 ± 0.14	0.11 ± 0.01
50	30	0	9.73 ± 0.84	5.46 ± 0.47	57 ± 5	5.3 ± 0.3	1.28 ± 0.11	0.11 ± 0.01
0	30	1	10.90 ± 0.62	8.00 ± 0.46	73 ± 4	Nil	2.76 ± 0.16	0.17 ± 0.01
0	30	0	7.66 ± 0.54	4.85 ± 0.34	63 ± 4	Nil	1.72 ± 0.12	0.10 ± 0.01

[a]Yield in grams of PHA per gram of residual non-PHA biomass.

1.3, and 1.4 times higher, respectively, than that of the 100 mL of culture volume. The consumption of glucose and organic acids was much faster in the better-aerated 50 mL culture conditions (data not shown). The PHA % in the cell mass and HV mol% were similar regardless of the culture volume (Table 1). These results coincide with the literature, which reported a 1.7 times higher amount of polymer (PHB) from beet molasses medium when a lesser medium volume was used in the shake flask culture of *A. vinelandii* [14].

Effect of Yeast Extract on PHA Production

The addition of 1 g/L of yeast extract to the 50% SWW medium containing 30 g/L glucose did not improve the level of cell growth (9.40 g/L with the yeast extract vs. 9.73 g/L without the yeast extract) and PHA production (5.48 g/L with the yeast extract vs. 5.46 g/L without the yeast extract) (Table 2). The rate of PHA production was also similar (0.11 $g \cdot L^{-1} \cdot h^{-1}$) regardless of yeast extract supplementation (Table 2). This result is different from that reported for beet molasses, in that yeast extract supplementation stimulated the formation of PHA as well as cell growth [16]. However, there was an increase in cell growth and PHA production when a 30 g/L glucose medium without SWW was used as the experiment. Yeast extract supplementation to the glucose medium increased the level of cell growth (from 7.66 to 10.9 g/L), PHA production (from 4.85 to 8.00 g/L), and rate of PHA production (from 0.10 to 0.17 $g \cdot L^{-1} \cdot h^{-1}$) (Table 2).

The difference between the two media on the effect of yeast extract supplementation appears to be due to the influence of the yeast extract on the C:N ratio in the medium. The C:N ratios of glucose-supplemented 50% SWW medium and glucose medium without SWW were calculated. In the glucose-supplemented 50% SWW medium, the amount of carbon and nitrogen were 1,240 and 53 mM, respectively, resulting in a C:N ratio of 23:1. This C:N ratio is similar to the optimal value (22:1) reported in the literature [16], and explains why the additional yeast extract did not promote cell growth or PHA production in the glucose-supplemented 50% SWW medium.

Table 3. Effect of mineral salts on the production of PHA in SWW medium supplemented with 30 g/L of glucose

SWW (%)	Mineral salts	DCW (g/L)	% PHA (dry wt)	PHA (g/L)
50	w/o	8.11 ± 0.71	60 ± 5	4.87 ± 0.43
50	w/	9.40 ± 0.96	58 ± 6	5.48 ± 0.56
100	w/o	5.70 ± 0.52	50 ± 5	2.85 ± 0.26
100	w/	5.18 ± 0.75	31 ± 4	1.62 ± 0.23

On the other hand, the C:N ratio of the glucose medium without SWW was only 69:1 [16]. Under this highly nitrogen-limited condition, the supply of nitrogen from 1 g/L of yeast might promote significant cell growth and PHA production. The typical nitrogen content in yeast extract is 4.8 wt%, and 1 g/L of yeast extract supplies 3.4 mmol nitrogen. Therefore, supplementation of the yeast extract increased the C:N ratio in the glucose medium without SWW to 56:1.

These results suggest that the load of nitrogen compounds in waste water treatment facilities can be reduced significantly by *A. vinelandii* UWD. Considering that yeast extract also contains other important micronutrients, the experimental results of yeast supplementation indicate that SWW is a well balanced feedstock that can be combined with other carbon-rich waste materials for microbial fermentation.

Effect of Inorganic Salts Supplementation on PHA Production

The effect of supplementing inorganic salts was examined by comparing the level of microbial growth and PHA production with and without the supplementation of inorganic salts to glucose-supplemented (30 g/L of glucose) SWW (Table 3). Among the inorganic salts tested, ammonium chloride (NH_4Cl) was included as a nitrogen source, as described in Materials and Methods. The supplementation of inor ganic salts affected the production of PHA depending upon the dilution of the glucose-supplemented SWW (Table 3). For 50% SWW, salt supplementation increased the level 1.4,

Table 4. Comparison of the level of PHA production using various organic wastes as feedstocks

Feedstock	Microorganism	Culture	Additive	DCW (g/L)	PHA (g/L)	% PHA (dry wt)	HV content (mol%)	Reference
Beet molasses	*Azotobacter vinelandii*	Batch	None	7.46	4.70	63	Nil	14
			Valerate	7.54	4.90	65	16	
Acidogenic fermented starchy wastewater	*Alcaligenes eutrophus*	Batch	None	3.50	1.20	34	–	11
Municipal wastewater	Activated sludge	Batch	None	–	–	20	–	24
			Acetate	–	–	30	–	
Anaerobically fermentated olive oil mill effluent	Activated sludge	Batch	None	0.65	0.35	54	4.0	26
Hydrolyzed whey permeate	*Pseudomonas hydrogenovora*	Batch	None	10.6	1.27	12	Nil	18
			Valerate	12.0	1.44	12	19	
Acidogenic fermentated paper mill wastewater	Enriched PHA-accumulating organisms	Batch	None	2.00	0.96	48	53-69	25
Swine wastewater[a]	*Azotobacter vinelandii*	Batch	None	2.02	0.69	34	7.9	This study
			Glucose[b]	9.40	5.48	58	4.3	

ND, not determined.
[a]Two-fold diluted swine wastewater (50% SWW).
[b]30 g/L of glucose.

of cell growth by 16% from 8.11 to 9.40 g/L, and PHA production by 13% from 4.87 to 5.46 g/L. On the other hand, for the undiluted SWW, the supplementation decreased the level of cell growth from 5.70 to 5.18 g/L and PHA production from 2.85 to 1.62 g/L.

The difference in the effect of inorganic salts supplementation between the 50% SWW and undiluted SWW can be explained by the toxicity of the mineral salts rather than by the C:N ratio of the medium. Originally, 50% SWW had a C:N ratio of 48:1, and the supplementation of inorganic salts increased the ratio to 21:1, which is close to the optimal ratio of 22:1 [16]. This improved C:N ratio explains the better cell growth and PHA production after supplementing the 50% SWW medium with inorganic salts. For the undiluted SWW, the original C:N ratio of 31:1 was increased to 19:1 with inorganic salt supplementation. This new ratio (19:1) was closer to the optimum level (22:1) than the original C:N ratio (31:1). However, the level of cell growth and PHA production decreased. Therefore, it is believed that the decrease in cell growth and PHA production in the undiluted SWW was due to the toxicity from the excess mineral salts. For the undiluted SWW medium, the initial salinity increased to 6,500 ppm after supplementation with the inorganic salts. This high level of salinity possibly inhibited cell growth and PHA production. On the other hand, for the 50% SWW medium, there was no inhibition by the inorganic salts even though the supplementation increased the initial salinity from 2,700 to 3,800 ppm. This suggests that 3,800 ppm is the inhibitory salinity level for *A. vinelandii*. In the undiluted SWW medium supplemented with 30 g/L of glucose, the salinity decreased from 6,500 to 3,000 ppm after 72 h incubation [29]. The salinity of the 50% SWW medium supplemented with 30 g/L of glucose also decreased by 55% during fermentation [29]. This shows that mineral salts can be removed from the SWW while producing value-added materials, such as biodegradable plastics. Therefore, the load of inorganic salts can be reduced significantly if the process can be applied as a pretreatment in waste water treatment processes.

Comparison of PHA Production Using Various Organic Wastes

The level of PHA production by *A. vinelandii* using SWW was compared with that using other organic wastes, such as beet molasses, wastewaters, and whey (Table 4). It was reported that beet molasses is a promising feedstock for the production of PHA by *A. vinelandii* (dry cell weight and PHA content was 7.46 g/L and 63 wt%, respectively) because the substrate cost for PHA production was only one-third that using glucose [14]. However, valerate needed to be added in order to produce the polymer with a HV fraction [14]. Hydrolyzed whey was also reported to be a good feedstock for the growth of *Pseudomonas hydrogenovora* (dry cell weight 10.6 g/L) but the PHA content was limited to 12 wt%, and valerate was needed to obtain PHA with a HV content [18]. Compared to the above two feedstocks, the present results show that glucose-supplemented (30 g/L) SWW is a promising feedstock for *A. vinelandii* UWD to produce a comparable amount of biomass (9.40 g/L) with a high PHA content (58 wt%), and reasonable HV fraction (4.3 mol%). These results showed that SWW is an excellent waste material containing an almost self-sufficient level of nutrients for PHA production except for the carbon source. In this study, glucose was used as the carbohydrate source. However, for a large scale applications, the carbohydrate cost can be reduced by supplementing carbohydrate-rich-waste materials. Other reports using different types of waste water [11,24-26] produced relatively smaller dry cell weights (0.65~3.5 g/L) with a lower PHA content (34~48 wt%) (Table 4).

CONCLUSION

The production of PHA using organic wastes can be an environmentally-friendly and economical alternative to using commercial feedstock. Swine wastewater (SWW) is a promising feedstock for PHA production because it contains a variety of nutrients for microbial growth including nitrogen and minerals. In particular, SWW contains alkanoates with an odd number of carbon atoms, which are essential for copolymer production. This study examined the effect of supplementing glucose, yeast extracts and inorganic salts on the production of PHA using *A. vinelandii* UWD. With glucose (30 g/L) supplementation, the level of cell growth, PHA production, and copolymer (HV) fraction were increased up to 8.5 times, 7.0 times, and 10.8 mol%, respectively, depending upon the dilution ratio of SWW. However, yeast extract supplementation did not improve the level of cell growth and PHA production, and inorganic salts supplementation provided only marginal improvement. Overall, SWW contains a self-sufficient level of nutrients for the production of PHA with some carbon limitation. This process is expected to reduce the load of inorganic salts and nitrogen compounds in waste water treatment facilities significantly.

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REFERENCES

1. Aldor, I. S. and J. D. Keasling (2003) Process design for microbial plastic factories: metabolic engineeering of polyhydroxyalkanoates. *Curr. Opin. Biotechnol.* 14: 475-483.
2. Reddy, C. S. K., R. Ghai, Rashmi, and V. C. Kalia (2003) Polyhydroxyalkanoates: an overview. *Bioresour. Technol.* 87: 137-146.
3. Suriyamongkol, P., R. Weselake, S. Narine, M. Moloney, and S. Shah (2007) Biotechnological approaches for the production of polyhydroxyalkanoates in microorganisms and plants – a review. *Biotechnol. Adv.* 25: 148-175.
4. Choi, G. G., H. W. Kim, Y. B. Kim, and Y. H. Rhee (2005) Biocompatibility of poly(3-hydroxybutyrate-*co*-3-hydroxy valerate) copolyesters produced by *Alcaligenes* sp. MT-16. *Biotechnol. Bioprocess Eng.* 10: 540-545.
5. Choi, J. I. and S. Y. Lee (2004) High level production of supra molecular weight poly(3-hydroxybutyrate) by metabolically engineered *Escherichia coli*. *Biotechnol. Bioprocess Eng.* 9: 196-200.
6. Ryu, H. W., K. S. Cho, B. S. Kim, Y. K. Chang, H. N. Chang, and H. J. Shim (1999) Mass production of poly(3-hydroxybutyrate) by fed-batch cultures of *Ralstonia eutropha* with nitrogen and phosphate limitation. *J. Microbiol. Biotechnol.* 9: 751-756.
7. Ryu, H. W., S. K. Hahn, Y. K. Chang, and H. N. Chang (1997) Production of poly(3-hydroxybutyrate) by high cell density fed-batch culture of *Alcaligenes eutrophus* with phosphate limitation. *Biotechnol. Bioeng.* 55: 28-32.
8. Du, G. C., J. Chen, J. Yu, and S. Lun (2001) Feeding strategy of propionic acid for production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with *Ralstonia eutropha. Biochem. Eng. J.* 8: 103-110.
9. Park, C. H. and V. K. Damodaran (1994) Effect of alcohol feeding mode on the biosynthesis of poly(3-hydroxybutyrate-co-3-hydroxyvalerate). *Biotechnol. Bioeng*. 44: 1306-1314.
10. Park, C. H. and V. K. Damodaran (1994) Biosynthesis of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) from ethanol and pentanol by *Alcaligenes eutrophus*. *Biotechnol. Prog.* 10: 615-620.
11. Yu, J. (2001) Production of PHA from starchy wastewater via organic acids. *J. Biotechnol.* 86: 105-112.
12. Yu, J., Y. Si, and W. K. R. Wong (2002) Kinetics modeling of inhibition and utilization of mixed volatile fatty acids in the formation of polyhydroxyalkanoates by *Ralstonia eutropha*. *Process Biochem.* 37: 731-738.
13. Page, W. J., J. Manchak, and B. Rudy (1992) Formation of poly(hydroxybutyrate-co-hydroxyvalerate) by *Azotobacter vinelandii* UWD. *Appl. Envrion. Microbiol.* 58: 2866-2873.
14. Page, W. J. (1992) Production of polyhydroxyalkanoates by *Azotobacter vinelandii* UWD in beet molasses culture. *FEMS Microbiol. Rev.* 103: 149-158.
15. Cho, K. S., H. W. Ryu, C.-H. Park, and P. R. Goodrich (1997) Poly(hydroxybutyrate-co-hydroxyvalerate) from swine waste liquor by *Azotobacter vinelandii* UWD. *Biotechnol. Lett.* 19: 7-10.
16. Cho, K. S., H. W. Ryu, C.-H. Park, and P. R. Goodrich (2001) Utilization of swine wastewater as a feedstock for the production of polyhydroxyalkanoates by *Azotobacter vinelandii* UWD. *J. Biosci. Bioeng.* 91: 129-133.
17. Kim, S. W., P. Kim, H. S. Lee, and J. H. Kim (1996) High production of poly-β-hydroxybutyrate (PHB) from *Methylobacterium organophilum* under potassium limitation. *Biotechnol. Lett.* 18: 25-30.
18. Koller, M., R. Bona, E. Chiellini, E. G. Fernandes, P. Horvat, C. Kutschera, P. Hesse, and G. Braunegg (2008) Polyhydroxyalkanoate production from whey by *Pseudomonas hydrogenovora*. *Bioresour. Technol.* 99: 4854-4863.
19. Ho, I. C., S. P. Yang, W. Y. Chiu, and S. Y. Huang (2007) Structure and polymer form of poly-3-hydroxyal- kanoates produced by *Pseudomonas oleovorans* grown with mixture of sodium octanoate/undecylenic acid and sodium octanoate/5-phenylvaleric acid. *Int. J. Biol. Macromol.* 40: 112-118.
20. Choi, J. and S. Y. Lee (1999) High-level production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by fed-batch culture of recombinant *Escherichia coli*. *Appl. Environ. Microbiol.* 65: 4363-4368.
21. King, P. P. (1982) Biotechnology. An industrial view. *J. Chem. Technol. Biotechnol.* 32: 2-8.

22. Page, W. J. (1989) Production of poly-β-hydroxybutyrate by *Azotobacter vinelandii* strain UWD during growth on molasses and other complex carbon sources. *Appl. Microbiol. Biotechnol*. 31: 329-333.
23. Son, H., G. Park, and S. Lee (1996) Growth-associated production of poly-β-hydroxybutyrate from glucose or alcoholic distillary wastewater by *Actinobacillus* sp. EL-9. *Biotechnol. Lett*. 18: 1229-1234.
24. Chua, A. S. M., H. Takabatake, H. Satoh, and T. Mino (2003) Production of polyhydroxyalkanoates (PHA) by activated sludge treating municipal wastewater: effect of pH, sludge retention time (SRT), and acetate concentration in influent. *Water Res*. 37: 3602-3611.
25. Bengtsson, S., A. Werker, M. Christensson, and T. Welander (2008) Production of polyhydroxyalkanoates by activated sludge treating a paper mill wastewater. *Bioresour*. Technol. 99: 509-516.
26. Dionisi, D., G. Carucci, M. Petrangeli Papini, C. Riccardi, M. Majone, and F. Carrasco (2005) Olive oil mill effluents as a feedstock for production of biodegradable polymers. *Water Res.* 39: 2076-2084.
27. Moon, S. H., J. M. Park, H. Y. Chun, and S. J. Kim (2006) Comparisons of physical properties of bacterial celluloses produced in different culture conditions using saccharified food wastes. *Biotechnol. Bioprocess Eng.* 11: 26-31.
28. Lim, S. J., Y. H. Ahn, E. Y. Kim, and H. N. Chang (2006) Nitrate removal in a packed bed reactor using volatile fatty acids from anaerobic acidogenesis of food wastes. *Biotechnol. Bioprocess Eng.* 11: 538-543.
29. Cho, K. S., H. W. Ryu, and C.-H. Park (1996) Production of polyhydoxyalkanoates from swine wastewater. *J. Kor. Soc. Environ. Eng.* 18: 1259-1270.