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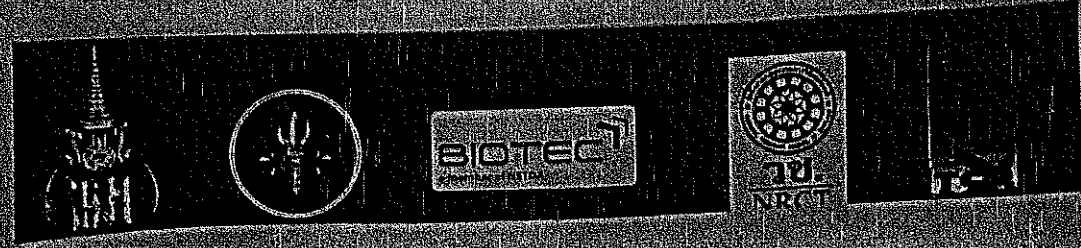
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Effect of pH and food-to-microorganism (F/M) ratio on PHB production from fermented cassava starch manufacturing wastewater (FCSW) by *Cupriavidus* sp. KKU38

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Effect of pH and food-to-microorganism (F/M) ratio on PHB production from fermented cassava starch manufacturing wastewater (FCSW) by *Cupriavidus necator* KKU38

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Abstract

Cassava starch manufacturing wastewater (CSW) was used as a substrate to produce polyhydroxybutyrate (PHB) by *Cupriavidus* sp. KKU38. The acidogenic fermentation process of CSW was firstly conducted to obtain the volatile fatty acids (VFAs). This research investigated the effect of pH (pH 6, 7, 8 and un-controlled) and food-to-microorganism (F/M) ratio (1, 2, 3, 4.5 and 6 C-mmol/C-mmol) on PHB production from fermented CSW by *Cupriavidus* sp. KKU38 at the flask scale. The results indicate that PHB production at pH 8 gives the maximum PHB content and yield of 61.47 % and 0.23 g PHB/g COD consumed, respectively. The enhanced F/M ratio at pH 8 for PHB production at the highest PHB content was 67.19 % while the yield was 0.25 g PHB/g COD consumed when the F/M ratio was enhanced to 2.0 C-mmol/C-mmol.

Keyword: *Polyhydroxyalkanoate (PHAs), Cassava wastewater, food-to-Microbial (F/M), Cupriavidus necator KKU38*

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1. Introduction

Plastics materials have become an integral part of contemporary life due to their versatile qualities of lightness, durability and resistance to degradation. These non-degradable plastics accumulate in the environment at a rate of millions of tons per year causing several problems. Recently, issues concerning the global environment and solid waste management have created much interest in the development of biodegradable plastics (Anderson and Dawes, 1990). Poly- β -hydroxyl butyrate (PHB) is the best characterized of all polyhydroxyalkanoate (PHA) storage materials found in many bacteria (Madison and Huisman, 1999). PHB has recently attracted much commercial interest as a plastic material because its physical properties are remarkably similar to polypropylene and polyethylene. It can be easily biodegraded in both aerobic and anaerobic conditions. However, the current cost of the PHB production is considerably more than that of the synthetic plastics. Therefore, techniques for cost reduction such as strain development, cultivation and downstream process strategy improvement as well as a search for inexpensive substrates for PHB production are required (Choi and Lee, 1999).

Several bacterial strains can utilize inexpensive carbon sources for the production of PHB. One of the best examples is the production of PHB by *Cupriavidus necator* (formerly *Wautersia eutropha*) which is a well known PHB producer that accumulates PHB up to 80% of the cell dry mass from various carbon sources (Reinecke and Steinbuchel, 2009). Volatile fatty acids can be utilized to produce PHB by various types of PHB producers such as *Alcaligenes eutrophus* (Yu, 2001) and *Ralstonia eutropha* (Du et al. 2001). *A. eutrophus* produce PHB from butyric acid while poly(hydroxybutyrate-hydroxyvalerate) was polymerized from propionic acid when the effluent from hydrogen fermentation of starch wastewater was used as substrate (Yu, 2001). Therefore, cassava wastewater can be used as a substrate for PHB production. Factors affecting biomass and PHB production are temperature, initial pH, substrate concentration, source of substrate while other factors affect efficiency of PHB production such as the food to microorganism (F/M) ratio. The microorganisms are measured as ppm or mg/L of MLTSS.

In this study, the effect of initial pH and F/M ratio on PHB production was investigated by using cassava starch. *Cupriavidus necator* KKU38, a newly isolated PHB producer from CSW was used as seed inoculum. The effects of pH and F/M ratio were examined in order to obtain the suitable conditions for PHB production and biomass.

2. MATERIALS AND METHODS

2.1 Cassava wastewater

Cassava starch manufacturing wastewater (CSW) was obtained from National Starch and Chemical Ltd., Kalasin Province, Thailand. The suspended solids in CSW were removed by simple gravity settling and the clear phase of CSW was used as a substrate for PHB production. Previous research reported that VFAs were an efficient substrate for PHB production, therefore, the CSW was fermented to obtain VFAs and subsequently used to produce PHB. The acidogenic fermentation was conducted in a 5 l laboratory glass bottle using anaerobic sludge (VSS of 16.25 g/l) from the UASB wastewater treatment plant of a beer factory (Khon Kaen Brewery Co. Ltd., Thailand) as a seed inoculum. The bottle was shaken at 150 rpm at room temperature for 48 h. Biogas produced was released via the port arranged at the top of the bottle. The suspended solids were removed and the clear phase of fermented CSW (FCSW) was used as a substrate for PHB production. The chemical characteristics of FCSW are shown in Table 1.

Table 1. Chemical characteristics of FCSW

Parameter	FCSW
pH	5.3
Total Chemical Oxygen Demand, - tCOD (mg/l)	17,200
Soluble COD, sCOD (mg/l)	9,000
Total nitrogen (mg/l)	71.74
Total phosphorus (mg/l)	81.11
Acetic acid (mg/l)	31.03
Butyric acid (mg/l)	16.3
Propionic acid (mg/l)	16.3

2.2 Microorganism preparation

Cupriavidus necator KCU38, a PHB producer, was isolated from CSW. It was grown in 100 ml E2 medium (Lageveen *et al.*, 1988) using 1000 mg C/l of acetate concentration as a C-source and incubated at 30°C, 150 rpm for one week. After one week, the culture was transferred into fresh E2 medium for 4 weeks. The culture medium was harvested by centrifugation at 10,000 rpm for 10 min and the pellets were re-suspended in 0.85% NaCl before use as seed inoculum for PHB production.

2.3 Cultivation medium

E2 medium (pH 7) was used as a cultivation medium in this study. NH_4Cl and KH_2PO_4 were used to adjust the nitrogen and phosphorus concentrations, respectively, in the culture medium to obtain the designed COD:N:P ratios of 100:1:11.

2.4 Batch experiments for effects of initial pH and food-to-microorganism (F/M ratio) on PHB production.

The batch-PHB production from FCSW by *Cupriavidus necator* KCU38 was conducted in a 250 ml Erlenmeyer flask with the working volume of 100 ml. The effects of initial pH in the substrate on PHB production were firstly investigated. Initial pH values of 7, 7.5 and 8 were used, and adjusted by addition of 1 M HCl or 1 M NaOH. The PHB production from FCSW without control of initial pH was conducted in parallel as a control. When the result indicated the initial pH that gave the maximum PHB content and PHB yield, it was used in the study on the effect of F/M ratios on PHB production.

The F/M ratio is an important parameter affecting the biological reaction process. In the following experiments, the initial concentration of biomass was fixed and a different amount of acetate was added to obtain a different F/M ratio. F/M ratio in the range of 1-6 C-mmol/C-mmol was investigated for PHB production. The initial concentration of biomass was fixed and a different amount of acetate was added to obtain a different F/M ratio. Thus, the different F/M ratio was represented as the different initial concentration of acetate in the batch. Sodium acetate was used to adjust for acetate concentration. A batch experiment was carried out in a similar manner as the study on the effect of substrate concentration described above.

2.5 Analytical procedure

Biomass concentration was determined by cell dry weight. The quantity of PHB was determined by the gravimetric method modified from (Kemavongse *et al.*, 2008). The residual cell concentration was calculated by subtracting the biomass concentration (g/l) and the PHB concentration (g/l) at the same sampling time. tCOD and sCOD concentrations were analyzed by using the dichromate closed-reflux method.

3. Results and Discussion

3.1 Optimization of operating conditions effect of initial pH

In order to evaluate the effect of initial pH on PHB production, a set of experiments was performed with pH control (pH 7, 7.5 and 8) and compared with the one without pH control. The PHB content and PHB yield are shown in Table 2. The results show that under uncontrolled pH conditions, the pH increased slowly within 24 h and gradually increased during cultivation from pH 4.72 to 9.25 while every controlled pH increased rapidly within 24 h and increased during cultivation to a maximum at pH 9.60 (data not shown). The maximum PHB content of 61.47% and PHB yield of 0.23 g PHB/g sCOD consumed were obtained at initial pH of 8 (Table 2), so an initial pH of 8 was used in the further experiments to study the effect of F/M ratio on PHB production by KCU38. A few published studies have described the effect of pH (in this range) on the rate and yield of

polymer accumulation, typically indicating a higher polymer production when the pH is controlled in the range from 8 to 9 (Marianna *et al.*, 2010).

Table 2. PHB production by *Cupriavidus* sp. KKU38 from non-diluted FCSW at various initial pHs.

pH value	Total COD remaining at (g/l)	Total COD consumption (g/l)	Maximum cell concentration (g/l)	Maximum PHB production (g/l)	Maximum PHB content (%)	Maximum PHB yield (g PHB/g sCOD consumption)
pH 7	2.08±0.04	6.16±0.02	2.20±0.12	1.28±0.06	58.45±6.21	0.21
pH 7.5	1.79±0.02	6.55±0.01	2.45±0.15	1.42±0.08	60.48±2.25	0.22
pH 8	2.19±0.03	6.21±0.05	2.33±0.05	1.43±0.05	61.47±3.11	0.23
Un-controlled	2.58±0.03	5.98±0.03	1.93±0.05	0.93±0.15	50.92±5.18	0.15

3.2 Optimization of operating conditions effect of F/M ratio.

As the results indicated that initial pH of 8 gave the maximum PHB content and PHB yield, it was used in the study on the effect of F/M ratios on PHB production. The initial concentration of biomass was fixed and a different amount of acetate was added to obtain a different F/M ratio. F/M ratio range of 1-6 C-mmol/C-mmol was investigated for PHB production. Johnson *et al.* (2009a) and Albuquerque *et al.* (2010) reported that batch conditions gave a very high PHA content production reaching 89% using acetate as carbon source. Therefore, acetate be used as a substrate for PHB production. The results indicate that an F/M ratio of 2 gave the highest maximum PHA content and PHB yield of 67.19% and 0.25 g PHB/g sCOD consumed, respectively (Table 3). Other F/M ratio gave PHB yields of 0.15-0.24 g PHB/g sCOD consumed. The lowest PHB yield of 0.15 g PHB/g sCOD consumed was obtained with an F/M ratio of 1. Lower biomass growth was associated with accumulations conducted with nutrient limitation (Table 3). The sCOD concentration decreased with increase in biomass and PHB concentration which indicates that the acetate as carbon source in the FCSW was consumed to produce biomass and PHB by KKU38 (Fig. 2). This study coincides with Dias *et al.* (2006) who reported that optimization of the mixed culture PHB production process has led to PHB contents of up to 65% with acetate as the substrate.

Table 3. PHB production by *Cupriavidus* sp. KKU38 from non-diluted FCSW at various F/M ratios.

Sample	Total COD remaining (g/l)	Total COD consumption (g/l)	Maximum cell concentration (g/l)	Maximum PHB production (g/l)	Maximum PHB content (%)	Maximum PHB yield (g PHB/g COD consumption)
F/M 1	1.51±0.03	6.60±0.03	2.02±0.04	1.02±0.06	50.44±5.09	0.15
F/M 1.5	2.05±0.02	6.07±0.01	2.40±0.01	1.47±0.05	66.16±2.28	0.24
F/M 2	2.60±0.03	6.12±0.05	2.23±0.05	1.50±0.06	67.19±3.26	0.25
F/M 3	1.86±0.02	6.25±0.03	2.33±0.10	1.43±0.08	61.46±3.06	0.23
F/M 4.5	2.46±0.03	6.46±0.01	2.13±0.16	1.40±0.06	65.95±5.86	0.22
F/M 6	2.85±0.02	5.76±0.05	2.07±0.26	1.22±0.04	59.71±8.28	0.21

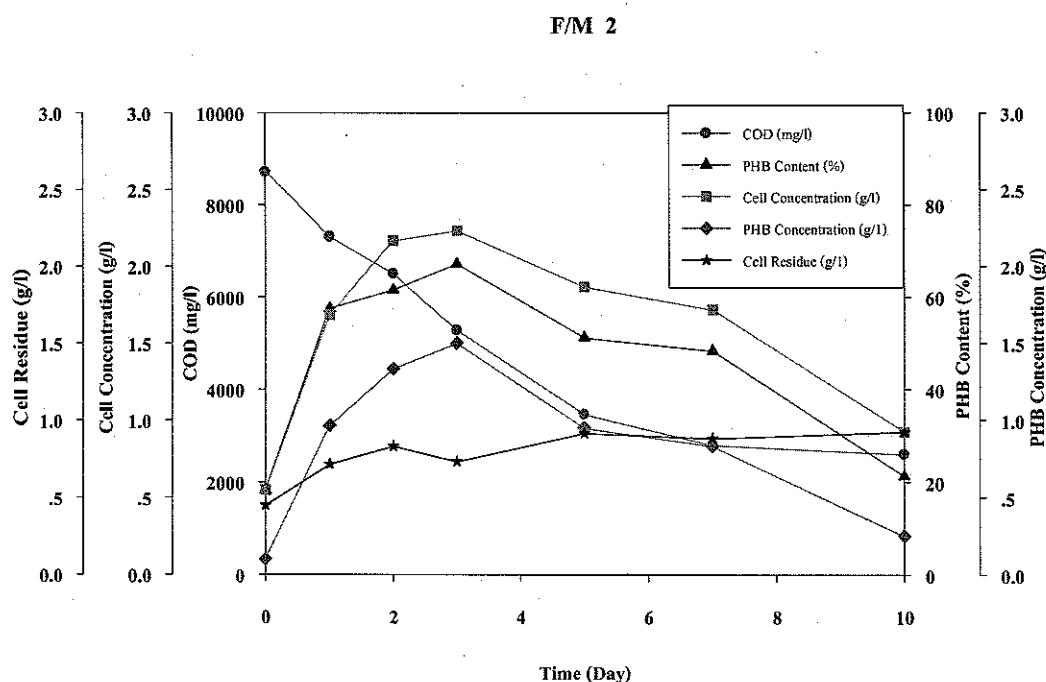


Fig 1. PHB production by *Cupriavidus necator* KKU38 from non-diluted FCSW at initial pH of 8 and F/M ratio at 2.

4. Conclusions

The PHB production from 9,000 mg-sCOD/l fermented cassava starch manufacturing wastewater by *Cupriavidus necator* KKU38 was optimized at initial pH of 8 which gave the maximum PHB content and yield of 61.47% and PHB yield of 0.23 g PHB/g sCOD consumed, respectively. Therefore an initial pH of 8 was used in the further experiment to study the effect of F/M ratio on PHB production by KKU38. Then research revealed the remarkable effect of F/M ratio on PHB production. Optimum conditions for

maximizing PHB production was an F/M ratio of 2. The PHB content and PHB yield of 67.19% and PHB yield of 0.25 g PHB/g sCOD consumed were obtained with F/M ratio of 2 respectively.

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