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Raw material resource for biodegradable plastic production from cafeteria wastes

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This study presents treatment performance of cafeteria waste and polyhydroxyalkanoate (PHA) production in two stage process through an up-flow anaerobic sludge bed (UASB) and sequencing batch reactor (SBR). COD removal efficiency was found as follows: SBR, 93; and UASB - SBR, 95.4%. In addition, 30% ammoniacal nitrogen (AN) was noted at high aerobic condition and phosphate (PO_4) removal efficiency increased at high anoxic conditions. Volatile fatty acid (VFA) intermediates such as acetic acid increased from 505 up to 4315 mg.l^{-1} in effluent of UASB reactor and successfully consumed in SBR for PHA production. Acetic acid concentration was reduced significantly in SBR from 4315 to 50 mg.l^{-1}. During this period, PHA production was increased to 68% over cell dried weight, confirming effectiveness of sequential UASB-SBR system in producing bioplastic from cafeteria waste.

Keywords: Cafeteria waste, Polyhydroxyalkonate (PHA), Sequencing batch reactor, Up-flow anaerobic sludge bed

Introduction

Plastics are xenobiotic compounds that are recalcitrant in natural decompositions[1,2]. Replacement of these non-biodegradable plastics were developed since 1970's for plastic industry[3,4]. Polyhydroxyalkanoate (PHA) has been attaining considerable attention as biodegradable plastics (or bioplastics) due to similar properties to thermoplastics and elastomers[5,6]. These properties completely degraded most of the consumer products to water and carbon dioxide (CO_2) upon disposal under various environments[7]. Most of the natural PHA produced from renewable carbon source was detected as polyhydroxybutyrate (PHB), since many of PHA-producer have a robust production in converting organic acid to hydroxybutyrates monomer (HB)[8-11]. However, application of PHA has been hindered by their high production cost, mainly due to the use of expensive carbon substrates and tedious production procedures (pure cultures)[12,13]. On the basis of achieving very high PHA content (70-75% wt/wt) in dry cell mass from microbial fermentation on pure glucose and organic acids, high production cost of PHA is mainly attributed to carbonaceous raw material (> 45%) and polymer recovery (> 26%)[14]. Bioplastics from renewable carbon source (cafeteria waste) could be rather new and promising because substrate can be easily utilized by the bacteria to form a biodegradable plastic[15].

Cafeteria waste is a wastewater produced in kitchen sinks and in hand basin in household or cafeteria without any input from toilets. The composition vary significantly in terms of time and place due to variations in water consumption in relation to discharged amount of substances[16,17]. Organic wastes from natural discharges are usually in complicated forms that cannot be directly utilized by PHA-producing species[18,19]. Both acid-producing and PHA-producing organisms are cultivated in a mixed culture, and acids released will be utilized in a 'fast' mode, called feast and famine regime. Feast period refers to the condition of plenty of food available for microorganisms, while famine period indicates that substrate is fully consumed and microorganisms depends on their intracellular storage to survive[20,21]. Microorganisms, which are able to quickly store and consume substrate in a more balanced way, have a strong competitive advantage over organisms without the

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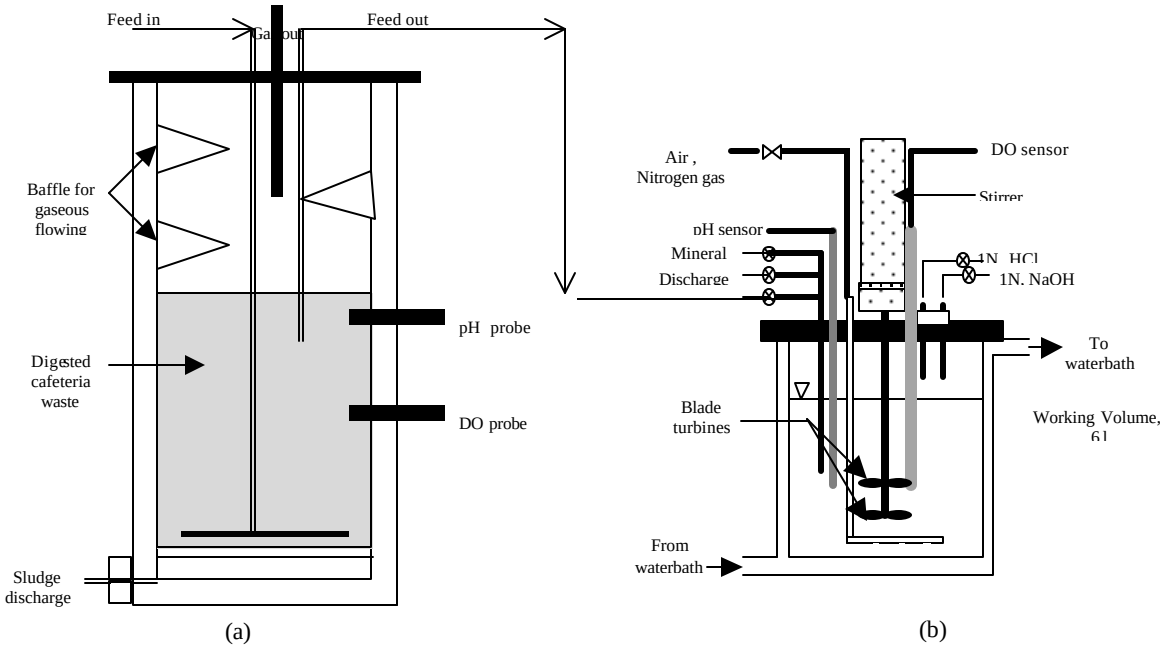

Fig. 1—Experimental set-up of combined (a) UASB and (b) Bio-PORec® reactor

capacity of substrate storage[22]. Consequently, a net population of PHA-producer in one operational cycle can produce PHA at higher rate.

This study presents production of biodegradable polymer material as well as to reduce chemical oxygen demand (COD) from cafeteria wastes, by using a two-stage treatment system comprising an up-flow anaerobic sludge bed (UASB) and sequencing batch reactor (SBR), especially to improve PHA production. In addition, nutrient removal efficiency (ammoniacal nitrogen, AN; phosphate, PO_4) and correlation between PHA and volatile suspended solids (VSS) in anoxic/aerobic system were also examined.

Experimental Section

UASB and Bio-PORec® System

This study was performed using two-stage biological reactor system. Initially, an UASB reactor (Fig. 1a) was used for pretreatment of cafeteria wastes. Effluent from UASB reactor was passed through a Bio-PORec® system for the production of PHA. Food scraps (cafeteria wastes) were collected from a local canteen and mixed with water (1:1, wt/wt) and blended to form slurry. Anaerobic digestion by UASB of cafeteria waste was accomplished at 35°C in a 10 l reactor with working volume of 9 l. A natural inoculum for food waste digestion (acidogenesis) was prepared in a slurry form for 30 d. Inoculum (30-40% v/v) was mixed with fresh slurry to start-up reactor and no deoxygenation was implied during the process. As soon as acid profiles achieved uniformity in 25 d, supernatant from UASB was used for batch production of PHAs in Bio-PORec® system (Fig. 1b). In general, Bio-PORec® system is a SBR with a working volume of 6 l and operated continuously in cycles of 6 h under alternating anoxic (nitrogen purging) and aerobic (air purging) conditions. Mixing was accomplished using a flat-blade stirrer of 1000 rpm and supernatant from UASB was transferred to SBR using peristaltic pump at a rate of 20-25 $ml.min^{-1}$. Temperature of Bio-PORec® system was maintained at 30°C via water jacket and anoxic conditions were assured by injecting nitrogen gas (N_2) to maintain dissolved oxygen (DO) at almost zero, while aerobic conditions were achieved by injecting DO at a flow rate of 2 $l.min^{-1}$. DO concentration was kept at 20% of air saturation during accumulation stage.

Chemical Analysis

Sample analysis included COD, AN, phosphate-phosphorus (PO_4-P), calcium (Ca^{2+}), sodium (Na^{+}), volatile fatty acids (VFAs), PHA, cell dried weight (CDW), VSS and ash constituents. Table 1 illustrates experimental conditions of the study. DO in reactor was measured online using a DO-electrode as a percentage of air saturation. Samples taken from reactor was immediately centrifuged and filtered using 0.45 µm filters, and analyzed using HACH DR4000 spectrophotometer. Quantification of CDW was performed using VSS and ash technique according to Dutch Standard[23].

Table 1—Experimental conditions during anoxic/aerobic fed-batch system

Experimental code	Anoxic h	Aerobic h	Feast/ famine min	Ratio of anoxic-aerobic, %	Operation
Ano-1	1	5	191/169	17 : 83	Temp., 25-28°C
Ano-2	2	4	127/233	33 : 67	pH, 7.00 ± 0.1
Ano-3	3	3	180/180	50 : 50	Air, 2.0 l/min
Ano-4	4	2	249/111	67 : 33	N_2, gas was purged during
Ano-5	5	1	-	83 : 17	anoxic for at least 0.5-1.0l/min

Table 2—Treatment profile of combined UASB-SBR system

Parameter	Raw Influent mg.l^{-1}	After UASB mg.l^{-1}	Overall Bio-PORec® Treatment, mg.l^{-1}
Chemical oxygen demand (COD)	1000-6530	650-4200	100 - 300
Acetic	505 - 2100	1750 - 4315	50 - 150
Propionic	32 - 425	435 - 1470	144 – 250
Butyric	65 - 955	375 - 1100	120 – 345
Lactic	398 - 3050	1500 - 5381	700 – 2430
Suspended solid	200-600	110 - 330	90 - 105
Volatile suspended solid (VSS)	105 - 205	52 - 103	20 - 50
Ammoniacal Nitrogen	35-78	20 - 46	10.5 – 18.1
Phosphate	54-100	11 - 21	20 – 19.5
Calcium	1.9-5.1	0.4 – 1.07	0 – 0.1
Sodium	89-196	13.4 – 56.8	0 – 0.1

PHB Assay

For PHB determination, samples were placed in 10 ml tubes and formaldehyde was added to stop all biological activities. Samples were washed with 5 mM of KH_2PO_4 buffer (pH 7) and centrifuged for 10 min at 10,000 rpm. Solid-free residuals were dried using freezer-dryer for 48 h and weighed. Dried biomass was determined by extraction, hydrolyzation and esterification using hydrochloric acid, 1-propanol, and dichloroethane at 100°C and 2 ml of benzoic acid was used as an internal standard throughout the procedure. It was followed by adding chloroform (2 ml), to eliminate any organic residuals. Mixed PHB, biomass and solvent were then digested at 100°C for 2 h using digester reflux. A 3 ml of distilled water was added into each bottle and placed in a shaker for mixing. Samples were then centrifuged and 1 µl was injected into a gas chromatograph (GC). PHB concentration was calculated as a percentage of total biomass dry weight (% PHB) and converted to g.l^{-1} or C-mM. A pure PHB was obtained by dissolving polymer in chloroform and precipitation with propanol. VFA intermediates (acetic, propionic, butyric) were measured with GC and a flame ionization detector (FID) with direct injection of acidified aqueous samples (pH 2-3) into a Supelco fused-silica capillary column (ø 0.25 mm x 25 m). Lactic acids was quantified using high pressure liquid chromatography (HPLC) equipped with a C_{18} column and ultra-violet (UV) diode-array detector. Mobile phase was 0.2% wt H_3PO_4 aqueous solution and monitored at a UV wavelength of 210 nm.

Results and Discussion

Process Performance

Food particles and raw material fluids from kitchen sinks are sources of solid material (35% household combustible waste), which contain[24,25]: moisture, 75-85%; and volatile solids, 85-95%. Since, kitchen waste consist high carbon-chain (COD) and low nutrient concentration, thus, these components were expected to be useful for PHA production[26]. Pretreatment of cafeteria waste was accomplished to enhance concentrations of VFA intermediates (acetic, propionic, butyric). VFAs produced in UASB reactor were pumped to Bio-PORec ® to improve PHA production. COD removal efficiency (95.4%) was observed in combined UASB - Bio-PORec® system (Table 2). SBR itself showed up to 93% COD removal efficiency and 35.7% COD removal efficiency was observed in UASB reactor, confirming less organic carbon reduction in anaerobic reactor compared to SBR system. Acetic acid concentration was

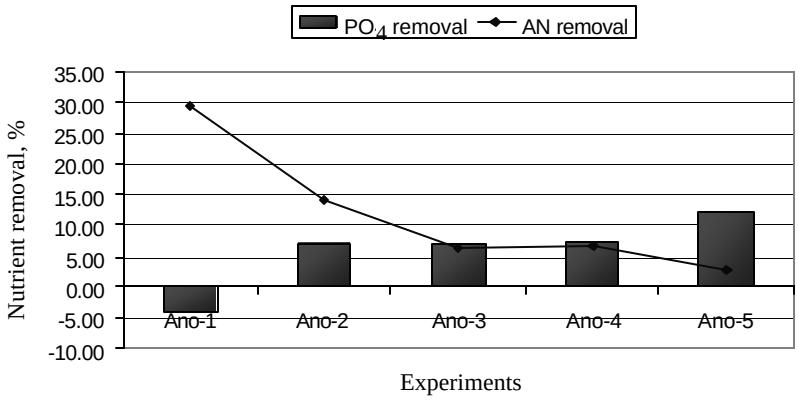

Fig. 2—Nutrient removal at different anoxic/aerobic conditions

increased from 505 up to 4315 mg.l^{-1} (Table 2) in effluent of UASB reactor. Propionic, butyric and lactic acid concentrations were also substantially increased in anaerobic reactor. VFAs produced in UASB reactor were successfully consumed in Bio-PORec® system for PHA production. Acetic and propionic acids were major carbon source for Bio-PORec® as most of PHA productions were derived from degradation of these two organic acids.

Feast and Famine Period

During substrate feeding period, DO decreased unexpectedly (data not provided) to a maximum rate due to substrate utilization (exogenous respiration). After substrate depletion, DO increased gradually and these changes were defined as famine periods, when microorganisms recover energy-substrate from their intracellular storages (PHA and glycogen). In order to increase PHA production, end of feast period was performed for each cycling period. DO profile was essentially similar to feast period; however it fluctuated in famine stage. Feast and famine phase was also occurred during transient of anoxic/aerobic condition, demonstrating that both NO_3 and O_2 have been used as electron acceptor (to accelerate substrate uptake).

Nutrient Removal

Nutrient removal profiles (Fig. 2) show that 30% AN removal was at Ano-1, and removal rate reduced to 3% at Ano-5, demonstrating high aerobic conditions had improved AN removal rate (anoxic/aerobic ratio of 17:83, Table 1). PO_4 removal efficiency (Fig. 2) depends on energy metabolisms[27] (ATP/NADP) and their removal efficiency were generated under high anoxic conditions (anoxic/aerobic ratio of 83:17, Table 1). Oxygen (O_2) concentration played important role in ammonification reaction with a greater increment of AN removal at high aerobic condition (Ano-1, Table 1). Typically, AN was assimilated from oxidation of substrate under aerobic conditions. However, presence of O_2 could not increase PO_4-P removal rate (Fig. 2). During feast period, biomass was limited and polymer storage was developed with the presence of excess external substrate. Once external substrate was completely consumed, stored polymer was used as an energy source for growth. In general, microorganisms utilize PO_4 (stored as Poly-P) for energy production and growth, although there is no external substrate are available[28]. Therefore, in current study, PO_4-removal efficiency may have been affected by high PHA production in the system.

Specific Production and Substrate Uptake Rate

Maximum ratio on production over substrate uptake rates ($q_p/-q_s$) was obtained at Ano-2 and decreased with increasing anoxic conditions (Fig. 3). The whole fraction rate of $q_p/-q_s$ depicted at more than 1 g PHB per g of VFA consumed. Apparent discrepancy is probably linked to conversion of substrate to PHA because it does not depend on VFA concentration. Presence of slow degradable substrate in cafeteria waste may have affected conversion efficiency of substrate to PHA. However, cafeteria waste did not show relationship between slow degradable substrate to PHA production[29,30]. Lowest ratio of $q_p^{feast}/-q_s^{feast}$ depicted at Ano-4 with 1.09 g $PHA_{produced}$/g $VFA_{consumed}$; however, their specific production and substrate uptake rate were high at longer anoxic conditions (4 h). This is contradictory to reported[31] findings, where aerobic uptake rates were found three times higher when compared with anoxic uptake rates. However, data of anoxic and aerobic substrate uptake with biomass grown under alternating anoxic and aerobic phases, as in real nitrogen removal

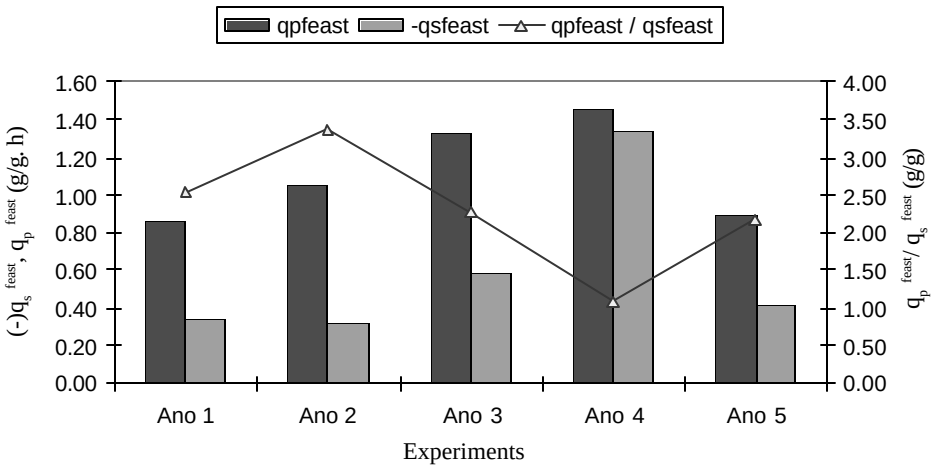

Fig. 3—Specific production and substrate uptake rate during feast period at different anoxic/aerobic conditions

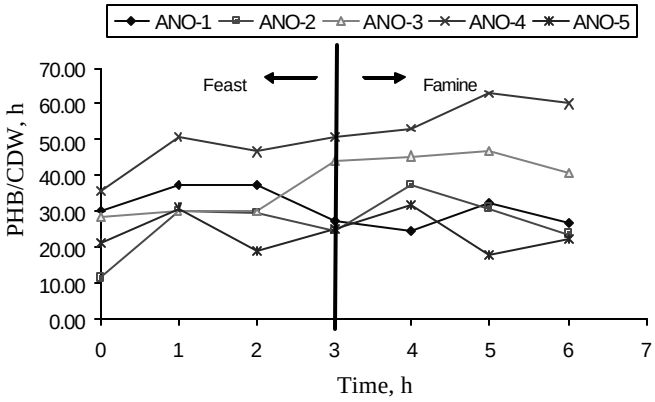

Fig. 4—PHA per CDW profile during feast and famine period during one cycle fed-batch system

plants, is still lacking[7]. In addition, ratio of $q_p^{feast}/ -q_s^{feast}$ implies that growth rate of active biomass present in the medium is not constant. In general, ratio of PHB per active biomass produced in feast period will vary according to the biomass growth[32]. As a result, this leads to a varying biomass composition of total biomass formed in feast period.

PHA Production

PHA is produced from VFAs and concurrently contributes to VSS increment. PHB production typically increased as 1.5, 2.0, 3.0, 4.0, 6.0 to 7.9 $g.l^{-1}$ when VSS in anoxic/aerobic condition increased from 5.8, 6.0, 6.3, 6.5, 7.7 to 8 $g.l^{-1}$, respectively, confirming a significant correlation between PHB and VSS, which stimulated from total biomass (PHA + VSS + residual SS). Highest PHB production occurred at Ano-4 (68% PHB/CDW) for a cycle of 6 h at different anoxic/aerobic conditions (Fig. 4). At appropriate anoxic period, initial PHB will have a tendency to accumulate higher polymer. Distribution of consumed PHB over anoxic/aerobic phases of famine period was based on the availability of electron acceptor (O_2 or NO_3) on both phases. Therefore, degradation of PHB during famine period was not clearly obtained, and inferred that feast and famine phases will not affect consumption of PHA, even at exhaustion of substrate.

Conclusions

There is a great potential for cafeteria waste as carbon source for the production of biodegradable plastic. Combined UASB-SBR system showed high COD and nutrient removal efficiency and effectively converted substrate to PHA. Conversion of organic acid to high PHA production can be controlled by O_2 and PO_4 concentrations. In addition, this study showed that high aerobic condition was appropriate for AN removal due to nitrification process; while PO_4 removal occurred at anoxic conditions. Ano-2 phase was found to be the most favourable condition for production of high PHA.

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