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Semi-Continuous and Continuous Anaerobic Treatment of Palm Oil Mill Effluent for the Production of Organic Acids and Polyhydroxyalkanoates

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Abstract: The aim of this study was to improve organic acids production in semi and continuous anaerobic treatment of Palm Oil Mill Effluent (POME) using a locally fabricated 50 L Continuously Stirred Tank Reactor (CSTR). The organic acids obtained were then used for polyhydroxyalkanoate (PHA) production by *Ralstonia eutropha* ATCC 17699 in a 2 L bioreactor. The conditions used in the anaerobic treatment were controlled pH 6.5, sludge to POME ratio at 1:1 and Hydraulic Retention Time (HRT) of 4 days. The organic acids obtained were about 15 g L^{-1} at steady state for both treatments and the organic acids yield (based on BOD) was 58.3%. The selected organic acids obtained were acetic (51.5%), propionic (27%) and butyric (21.5%) acid. The recovery of the clarified and concentrated organic acids from the treated POME was made using a two-steps evaporation process. The clarified organic acids (distillate) obtained were comprising of 44.6, 20.1 and 22.5 g L^{-1} acetic, propionic and butyric acids, respectively with a recovery of 76%. The organic acids collected were then used for polyhydroxyalkanoates accumulation by *Ralstonia eutropha* ATCC 17699 using pH stat fed-batch fermentation under nitrogen limitation of C/N 40 in a 2 L fermenter. The highest PHA concentration of 11.4 g L^{-1} (>90% w/w) was achieved in this process.

Key words: Palm oil mill effluent, anaerobic treatment, organic acids, polyhydroxyalkanoates, distillation

INTRODUCTION

Palm oil industry is one of the main agro-industries in Malaysia. There are currently about 265 active palm oil mills in Malaysia. Beside Crude Palm Oil (CPO) as the main product, a lot of wastes are produced particularly in the form of Palm Oil Mill Effluent (POME). POME is generated from three major sources, namely sterilizer condensate, hydrocyclone waste and separator sludge. POME is a thick brownish liquid contains large amounts of COD (50,000 mg L^{-1}) and BOD (25,000 mg L^{-1}), 4-5% total solid (including 2-4% suspended solid, mainly debris from the fruits (Najafpour *et al*., 2006). Therefore, the palm oil mill industry in Malaysia has been identified as the one that produces the largest pollution load into the rivers throughout the country. Biological treatment of POME has been widely studied. The common practice of treating POME is by using ponding system which has particular disadvantages such as a long Hydraulic Retention Time (HRT) of 45-60 days, bad odor and difficulty in collecting biogas which can cause negative effects on the environment (Tramsek *et al*., 2007). Considering the highly organic character of POME, an anaerobic process would be the most

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suitable for POME treatments. We have carried out POME treatment using a 500 m^3 closed anaerobic digester which achieved the treatment efficiency of more than 95% COD removal during the start-up operation at 17 days HRT (Yacob *et al*., 2006).

During the anaerobic process, organic pollutants are degraded by a consortium of many microbial strains. Acidogenesis is one of the steps involved in anaerobic digestion where acidogenic (or fermentative) bacteria digest the hydrolysis products to simple organic compounds, predominantly volatile fatty acids (Appelsa *et al*., 2008). At high loading rates, rapid turnover and accumulation of organic acids will occur and the degradation of organic acids into methane is inhibited. Hydraulic retention time is one of the major factors in selecting the acidogenic bacteria to be dominant in the liquid waste in continuous anaerobic treatment. Since, the ultimate goal of wastewater management is towards zero discharge, the best waste water treatment scheme is inevitably a treatment that allows 100% reuse and recycling of the water or the bioconversion of the waste into valuable products. It was shown that 10 g L^{-1} organic acids were produced in continuous anaerobic treatment of POME at five days retention time (Nor Aini *et al*., 1999). Thus, this study was conducted to improve the organic acids yield from POME in continuous and semi-continuous anaerobic treatment in a large scale (50 L reactor). Organic acids produced during the anaerobic treatment could be recovered and used as fermentation feedstock for value-added products such as polyhydroxyalkanoates (PHA). The price of PHA will be substantially reduced by the use of more inexpensive carbon source such as agricultural or food industrial wastes. We have developed a process for the treatment of POME coupled with PHA production utilizing organic acids from treated POME (Hassan *et al*., 1997).

Besides optimization of the fermentation process for a high yield of products, improving the downstream processing is also of great interest. In that regard, a large number of studies were proposed such as liquid-liquid extraction, ion exchange, adsorption, electrodialysis and other membrane separation techniques (Bouchoux *et al*., 2006). In this study, the recovery of clarified organic acids from treated POME by using a two steps distillation process was carried out. The clarified acids were then subjected to the PHA accumulation stage by *Ralstonia eutropha* in a 2 L bioreactor.

MATERIALS AND METHODS

Substrates

POME and its sludge were obtained from FELDA Serting Hilir palm oil mill in Negeri Sembilan, Malaysia and stored in a cold room at 4°C before use. The sludge was sieved using 1.5 mm mesh to remove coarse particles. The characteristics of POME used in this study are shown in Table 1.

Anaerobic Treatment

Anaerobic treatment of POME was carried out to produce organic acids under non-sterile conditions in a fabricated 50 L bioreactor with dimensions of 71 cm height and 30 cm diameter. The main body of the vessel was made from transparent fiber and there were four baffles equipped at the side of the bioreactor. The bioreactor was equipped with two settling tanks, a product tank and a feeding tank. Mixing was accomplished by using a motor (G.K Heller model Glascol). The temperature

Table 1: Characteristics of raw POME used in this study

Parameters	Average
pH	4.400±0.2
BOD (g L^{-1})	22.00±2
COD (mg L^{-1})	88.00±8
Total solid (mg L^{-1})	55.00±15
Total suspended solid (g L^{-1})	45.00±15
Ammonical nitrogen (g L^{-1})	0.250±0.05
Formic acid (g L^{-1})	1.000±0.7
Acetic acid (g L^{-1})	1.700±2.0
Propionic acid (g L^{-1})	Not detected
Butyric acid (g L^{-1})	Not detected

was controlled at 30°C using B. Braun Termomix® BU and the pH was controlled at 6.5. Semi-continuous and continuous treatments of POME were conducted at 4 days Hydraulic Retention Time (HRT). POME was fed for three times a day for the semi-continuous treatment.

Recovery of Organic Acids

The recovery of organic acids from the treated POME involved two steps. After removing the suspended solid by centrifugation, the treated POME was concentrated using a rotary vacuum evaporator (Eyela SB-651) up to 10 folds at a vacuum pressure of 40 mmHg and at 50°C for the first step. Then, the concentrated POME was collected for a further recovery process. A 400 mL of concentrated treated POME was mixed with 50 mL 18.76 M sulphuric acid (ratio 8:1) and evaporated at a vacuum pressure of 40 mmHg and at a temperature of 90°C for the second step. The clarified organic acid solution was then collected in a flask condenser and kept for further use.

PHA Production

The clarified organic acids collected were used as the carbon source for PHA production by *Ralstonia eutropha* (ATCC 17699) in pH-stat fed-batch fermentation under sterile condition, at a controlled pH of 7 and at 30°C. The fermentation was carried out in a 2 L bioreactor containing minimal medium sterilized for 20 min at 121°C. The medium contents in g L^{-1} were Na_2HPO_4, 3.80; $MgSO_4.7H_2O$, 0.20; $Fe(NH_4)_2(SO_4)_2.6H_2O$, 0.12; KH_2PO4, 1.5; K_2HPO_4, 1.8; acetic acid 1.0. The samples (fermentation broth) were collected daily and kept at -20°C prior to analysis.

Analytical Methods

The cell concentration was determined by measuring the optical density of the culture broth with a spectrophotometer at 600 nm. The OD value was then converted to dry cell weight (g L^{-1}) using a relationship obtained previously. The PHA and organic acids were measured by HPLC (Shimadzu LC-10AS with UV-VIS detector SPD-10A) with 4 mm sulphuric acid as the mobile phase at a flow rate of 0.6 mL min^{-1}. BOD, COD, dissolved solid and Total Kjeldahl nitrogen, total solid and total suspended solid of the effluent were determined based on the Standard Methods (APHA, 1998).

RESULTS AND DISCUSSION

Organic Acids Production in Semi-Continuous and Continuous Anaerobic Treatment of POME

Raw POME contains high suspended solid, BOD and COD load as shown in Table 1, which require a long retention time for satisfactory digestion. However in this study, the main purpose was the bioconversion of POME into organic acids (acidogenesis). In anaerobic digestion, the acid-forming and the methane-forming microorganism differ widely in terms of physiology, nutritional needs, growth kinetics and sensitivity to environmental conditions (Chen *et al*., 2008). Therefore, suitable parameters for acidogenesis should be controlled such as temperature, pH and HRT. In this study, methanogenesis was assumed to be inhibited due to the low pH (pH 6.5) being applied. The optimum pH for hydrolysis and acidogenesis had been reported were between pH 5.5 and 6.5. The growth rate of methanogens is greatly reduced below pH 6.6. Moreover, organic acids produced in the process of anaerobic digestion are also capable in inhibiting methanogenesis at high concentrations (Ward *et al*., 2008). Figure 1a shows the organic acids production in a continuous anaerobic treatment of POME. The continuous feeding of POME was started after day one of the fermentation. It was noticed that the production of organic acids fluctuated at the beginning of the treatment indicating that the system was unstable. However, a steady state was achieved after the fifth retention time on day 20. During the steady state, the average total organic acids produced was 15.04 g L^{-1} with acetic acid as the main acid (7.52 g L^{-1}) followed by propionic acid (4.47 g L^{-1}) and butyric acid (3.05 g L^{-1}). Lactic and

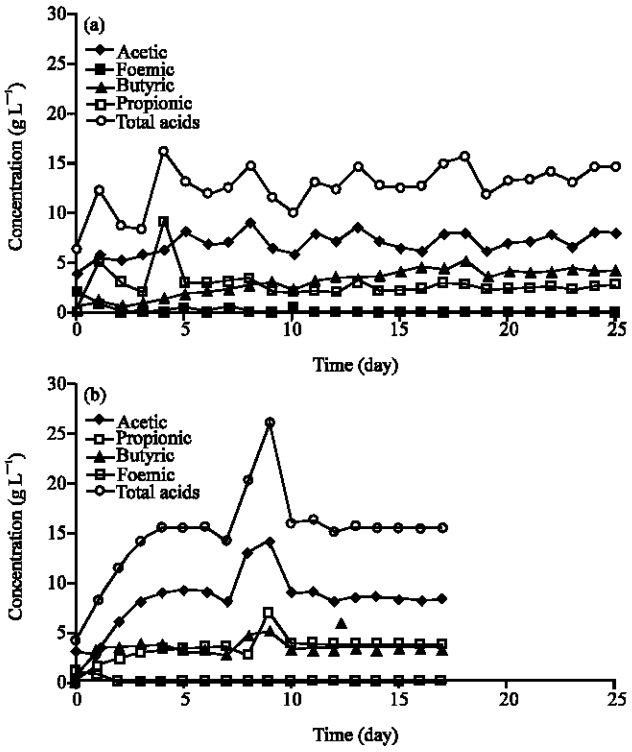

Fig. 1: Organic acids production during anaerobic continuous treatment of palm oil mill effluent in 50 L bioreactor (30 °C, pH 6.5), (a) continuous anaerobic treatment and (b) semi-continuous anaerobic treatment

formic acid were also measured but these acids were almost undetected throughout the treatment. Acetic acid is the major fermentation acids from the acidogenesis of organic matter by a mixed microbial population and it has quite a consistent concentration. Propionic acid or butyric acid however may be the second complementary acid depending on the organic loading rate, microbial population and the reactor design and operation (Yu, 2002). Salmiati *et al*. (2007) reported that the total organic acids produced were in the range of 5 to 8 g L^{-1} in the anaerobic treatment of POME using a continuous flow anaerobic reactor.

Figure 1b shows the profile of organic acids production from POME in semi-continuous anaerobic treatment and the steady state was achieved after the second residence time. The peak occurred at day 9 might be due to the instability of the system at that fermentation time before it reached steady state. The steady state achieved in both the experiments was much earlier when compared to the earlier studies by Nor Aini *et al*. (1999) and Phang *et al*. (2003). This was probably due to the controlled temperature at 30°C throughout the fermentation. When temperature changed or fluctuated, the temperature-susceptible bacteria could be shocked and the whole digestion process would be affected (Cha *et al*., 1997). The average total organic acids produced in the semi continuous anaerobic treatment was almost similar to that of the continuous anaerobic treatment which produced 15.3 g L^{-1} of total organic acids at the steady state. Acetic acid was the predominant acid (8.1 g L^{-1}) produced followed by propionic acid (3.7 g L^{-1}) and butyric acid (3.5 g L^{-1}). Initially, the concentration of formic acid was 1.5 g L^{-1} and it depleted at day 3 onwards. Therefore, it can be concluded that the semi-continuous system would offer a better and simpler way for handling the process.

Besides organic acids, there were other substances present in the effluent attributed to the metabolic intermediates of the process. Furthermore, a number of other soluble C1 to C4 compounds could be generated in small amounts such as organic acids, alcohols, aldehydes and ketones (Elefsiniotis and Oldman, 1994). This observation corresponded with the dissolved solid content in the effluent where the concentration of the dissolved solid was higher than the organic acids obtained.

Treatment Efficiency

Figure 2 shows the profile of the Total Suspended Solid (TSS) and Total Solid (TS) of the treated POME in the semi and the continuous anaerobic treatments. The values of TS and TSS gradually decreased during the fermentation and exhibited almost a constant values at the steady state. The average of the TS values for the semi and the continuous treatment were 50.3 and 44.7 g L^{-1}, respectively, while for the TSS the values were 15 g and 8 g L^{-1}, respectively for the semi and the continuous anaerobic treatments of POME. It should be noted that TSS value obtained at the end of the treatment was reduced by more than half (63%) as compared to the untreated POME.

The reduction of TSS and TS implying the degradation of POME occurred during the treatment. This process begins with the hydrolysis of complex organic compounds in the raw POME to more soluble intermediates. Throughout the process of acidogenesis, these intermediates are primarily broken down into organic acids and other monomer species (Salmiati *et al*., 2007). In general, a fraction of TSS which is not digestible is gradually accumulated in the bioreactor and causes reduction in the efficiency of the process.

The average of the COD and BOD values obtained for the semi-continuous treatment were 84000 g L^{-1} and 28100 mg L^{-1}, respectively during the steady state (data not shown). The high concentrations of the COD and BOD effluents were due to the organic acids, suspended solid and other residues in the treated POME. In this case, the COD reduction from POME was about 12.5% and the value was much lower as compared to other reports by Najafpour *et al*. (2006), Vijayaraghavan *et al*. (2007) and Zinatizadeh *et al*. (2007). However, for the purpose of organic acids production, the reduction of COD and BOD was not an indicator for the treatment efficiency. According to Banerjee *et al*. (1999), an increase in the organic acids content from the anaerobic digestion of organic wastes corresponded to the increase of the soluble COD; higher the percentage of suspended solid destruction, the better the settling properties of the clarifier.

Organic Acids Recovery

In this study, a two steps evaporation process was tested for recovering clarified organic acids solution. The organic acids were successfully concentrated almost 10 folds with 9.1% volume when

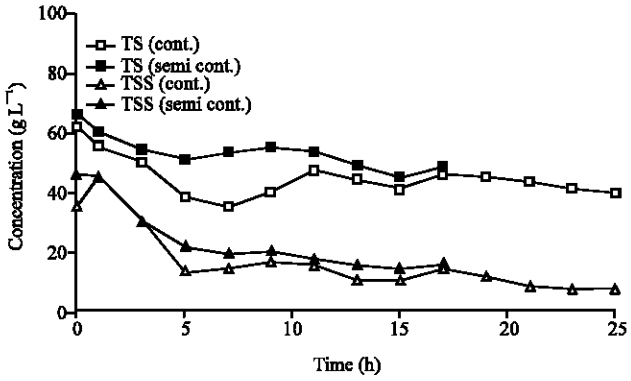

Fig. 2: TSS and TS profile during the anaerobic treatments of POME

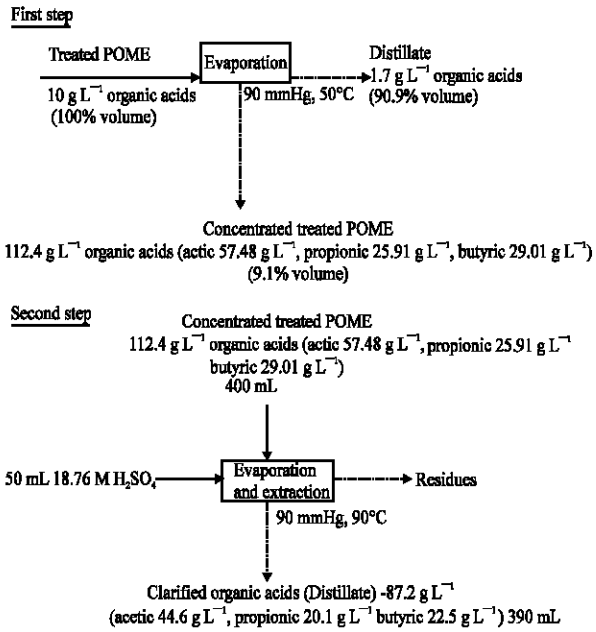

Fig. 3: Simplified process of clarified organic acids recovery from treated POME

compared to the volume of the original treated POME through an evaporation method for the first step (Fig. 3). It was noticed that the distillate contained a very low concentration of organic acids (1.7 g L^{-1}) with 90.1% volume. By adding concentrated H_2SO_4 into the concentrated treated POME and evaporating at ~90°C in the second step, a distillate comprising of acetic (44.6 g L^{-1}), propionic (20.1 g L^{-1}) and butyric (22.5 g L^{-1}) acids were obtained with a total concentration of 87.2 g L^{-1} and the acids recovery was 0.76 (g^{-1}). Figure 3 shows the whole process involved in the recovery of the clarified organic acids.

Addition of concentrated sulphuric acid in the treated POME resulted in the breaking of sodium from the organic acids thus reducing the boiling point of these organic acids. At this stage, the concentrated organic acids were evaporated and condensed leaving the salt (residue) in the flask. The distillate obtained which is the concentrated acids (87.2 g L^{-1}) was very clear and suitable to be used as a feeding substrate for microbial fermentation or for other purposes. It was reported that the organic acids generated by the anaerobic fermentation of wastes can be used as carbon sources for PHA production (Tsuge, 2002). The organic acids recovered in this study contained ~23% of propionic acid. The presence of propionic acid leads to the production of a copolymer of P(HB/HV). Dionisi *et al.* (2004) obtained a homopolymer of polyhydroxyvalerate (PHV) from propionate and a copolymer (34% HB and 66% of HV) from a mixture of acetate and propionate.

Polyhydroxyalkanoate Production

Figure 4 shows the PHA produced from *Ralstonia eutropha* using the clarified organic acids as carbon source. The results indicated that for the first 24 h of cell growth, Cell Dry Weight (CDW) was increased at the maximum level of 9.2 g L^{-1} with PHA content of 76% w/w and ammonium concentration was almost 1 g L^{-1}. During the PHA accumulation phase, substrate with C/N 40 was fed and the CDW was increased up to 12.5 g L^{-1} with a PHA concentration of 11.4 g L^{-1} at 192 h of fermentation. High PHA content was observed in the cells with over 90% (g^{-1}). *Ralstonia eutropha* accumulated a large amount of polymer up to 80% dry cell weight when nitrogen or phosphorus was

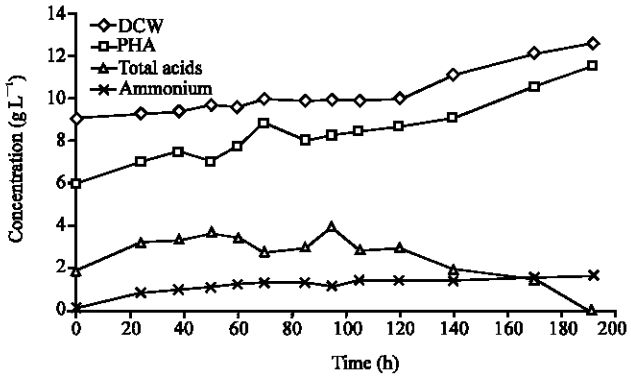

Fig. 4: PHA production by *Ralstonia eutropha* utilizing organic acids from palm oil mill effluent in pH-stat fed-batch fermentation

completely depleted (Khanna and Srivastava, 2005). Butyric and propionic acids were not detected throughout the fermentation process implying that they were consumed rapidly throughout the fermentation as compared to acetic acid. According to Ruan *et al*. (2003), butyric acid was better for PHA polymerization by *Ralstonia eutropha* when compared to acetic and propionic acids. When butyric acid was consumed by *Ralstonia eutropha*, it could be used as a carbon source for cell growth besides PHA accumulation. When used, butyric acid would undergo the β-oxidation pathway and directly converted to acetoacetyl-CoA instead of to acetyl-CoA then being converted to acetoacetyl-CoA. In the normal pathway for PHA synthesis, when a nitrogen source is deficient in the cell, the flux of acetyl-CoA to the TCA cycle decreases. The concentration of acetoacetyl-CoA then becomes greater in the β-oxidation pathway of butyric acid and the flux to the PHA synthesis pathway increases (Shimizu *et al*., 1993).

CONCLUSION

Organic acids production was improved in large scale (50 L CSTR) of semi and continuous anaerobic treatment of POME when conducted at pH 6.5, 30°C, 4 days HRT. Total organic acids of 15 g L^{-1} were produced during steady state for the both treatments. The clarified and concentrated acids (87.2 g L^{-1}) were successfully recovered using a two steps evaporation process with a recovery of 76%. *Ralstonia eutropha* ATCC 17699 was able to produce PHA using the recovered organic acids and accumulated PHA of 11.4 g L^{-1} with PHA content over 90% (w/w) in pH-stat fed-batch fermentation. The findings of this study would be useful for the subsequent commercialization of PHA production from POME.

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