



## Dark co-fermentation of skim latex serum (SLS) and palm oil mill effluent (POME) under thermophilic conditions for efficient biohydrogen production

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### Abstract

The aim of this study was to investigate the optimum mixing ratio of skim latex serum (SLS) to palm oil mill effluent (POME) for biohydrogen production under thermophilic temperature (55°C) by using thermophilic mixed cultures. Batch co-fermentations were carried out at various mixing ratios of SLS to POME (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45 and 50:50 (%v/v)) without supplementation of additional nutrients. The result shows that the optimum mixing ratio of SLS to POME was 75:25 (%v/v) with high hydrogen content, hydrogen production rate and hydrogen production yield of  $27.1 \pm 0.8\%$ ,  $19.6 \pm 0.3 \text{ mL H}_2/\text{d}$  and  $35.0 \pm 1.2 \text{ mL H}_2/\text{g-COD}$ , respectively. In addition, the hydrogen production yield achieved from co-fermentation was 1 and 5 times higher than that achieved from individual fermentations of SLS and POME, respectively. Propionate and butyrate were the major soluble end-products with concentrations  $38.20 \pm 0.63 \text{ mM}$  and  $24.35 \pm 0.00 \text{ mM}$ , respectively. Therefore this research work demonstrated significant feasibility of anaerobic dark co-fermentation of SLS and POME to enhance biohydrogen production. However, further nutrient optimization could be investigated in order to further increase hydrogen production yield.

**Keywords:** Biohydrogen production, Skim latex serum (SLS), Palm oil mill effluent (POME), Dark co-fermentation.

## 1. Introduction

Research in dark co-fermentation for hydrogen production from various organic substrates has much received attention in recent years, however many researchers utilize typical simple sugars or starch (1-5) which is not economically feasible due to their high cost. The new strategy of market-driven research is to focus on using cheap, organic waste-based feedstock, employing indigenous mixed cultures and improving the hydrogen production yield (6). Natural rubber (NR) latex is milky white or slightly yellowish opaque fluid of the rubber tree (*Hevea brasiliensis*). NR latex harvested from plantations needs to be processed soon after harvesting to maintain its quality. One of the major types of processed rubber is the preserved latex concentrate. Ammoniated field latex containing 30–33% rubber is concentrated by centrifuging to 60% rubber and skim latex (containing 4–6% rubber) using high speed centrifuges. The skim latex (contains about 0.8% ammonia) is coagulated with 98% sulfuric acid to recover rubber. The skim serum produced after coagulation of rubber is stored in a separate trap. It contains significant amount of non-rubber matters, including proteins, carbohydrates, sugars, carotenoids, organic and inorganic salts which are originated from the latex and very little amount of un-coagulated latex. These constituents are excellent substrates for the proliferation of microorganisms generating high biochemical oxygen demand (BOD) (7).

The production of palm oil as one of the major edible oils consumed in the world has increased tremendously in the last decade and is led by Malaysia, Indonesia and Thailand. However, the milling process produces a huge volume of palm oil mill effluent (POME). One ton of oil palm fruit approximately 0.87 m<sup>3</sup> POME is generated or 3.7 ton of effluent per ton of oil produced. Oil palm mill plant also generates large amount of solids wastes such as empty fruit bunch (EFB) (23%), mesocarp fiber (12%)

and shell (5%) for every ton of fresh fruit bunches (FFBs) processed in the mills. Thus, the utilized of POME has gained interest from many researchers due to the abundant amount generated in the mills.

Currently, most of latex concentrates plants and palm oil mill plants use SLS and POME as feedstock to produce biogas in Malaysia and Thailand (8). Hydrogen is one of the prototype alternatives renewable energy to fossil fuels. Hydrogen can be produced by many methods among that biological method by dark fermentation. This method is environmentally friendly and capable to utilize the organic waste to eliminate the pollution (9). Dark fermentation has various advantages, for example, high rate of bacterial growth, requires low energy input, no oxygen limitation problems and low costs (10, 11).

Various types of waste materials can be used as substrate for biohydrogen production such as palm oil mill effluent (POME) (8), starch-based materials (12), lignocellulosic materials (13), food wastes (14), dairy wastes (15), glycerol waste (16) and skim latex serum (17). Co-digestion of various organic wastes for energy production has several advantages compared to single substrate digestion such as improved biogas yield, economic advantages derived from the sharing of equipment, easier handling of mixed wastes, and synergistic effect (18, 19). Mixed culture is more appropriate for hydrogen production from non-sterilized organic wastes under mesophilic temperatures at 20 to 40°C (14, 15, 20) or thermophilic conditions at 50 to 60°C (21-23). Thermophilic operation may be suitable to meet legislation for treatment of feedstock containing pathogens. Additionally, thermophilic hydrogen production process was more efficient than the mesophilic one in both hydrogen production rate and yield (8, 24).

The current study aimed to determine the potential of using POME, wastewater from palm oil mill plant for co-digestion with SLS to produce hydrogen via mixed cultures fermentation.

## 2. Materials and methods

### 2.1 Anaerobic seed sludge

The anaerobic seed sludge used in this experiment was obtained from lab-scale upflow anaerobic sludge blanket reactor (UASB) producing hydrogen from SLS. The sludge was pretreated at 121°C for 15 minutes to remove methanogenic bioactivity. The sludge was enriched with synthetic medium according to Angelidaki and Sanders (25) with 10 g/L of sucrose and operated under thermophilic conditions (55°C) in 150 mL of serum bottles with 50 mL of working volume.

### 2.2 Skim latex serum (SLS)

The fresh raw SLS was collected from Chana Latex co, Ltd., Songkhla, Southern Thailand. The pH of mild yellow raw SLS was 4.83±0.01. Raw SLS was kept at 4°C prior using in order to minimize self-biodegradation and acidification. Some chemical and physical characteristics of SLS are given in table 1.

### 2.3 Palm oil mill effluent (POME)

The fresh raw POME was collected from the receiving tank of Palm Pattana Southern Border Co, Ltd., Pattani Southern Thailand. Raw POME has brown color, pH 4.68±0.00, a temperature of 70-80°C. The POME was fully characterized, as presented in Table 1, and kept in cold room at 4°C prior experimental conducting.

### 2.4 Batch hydrogen production from SLS and POME

Co-digestion of SLS with POME was tested at different mixing ratios of SLS to POME (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45 and 50:50 (%v/v)). The assay was conducted as batch cultivations in 150 mL serum bottles. In each bottle, 15 mL of inoculum and 35 mL of substrate/water mixture were added. The mixtures were then purged with N<sub>2</sub> (100%) to ensure anaerobic conditions. Afterwards the bottles were closed with butyl stoppers and placed in a 55°C incubator for 8 days. Hydrogen production in the headspace of the vials was monitored. The headspace gas was collected for hydrogen determination daily.

**Table 1.** Chemical and physical characteristics of SLS and POME

Characteristics	SLS	POME
pH	4.83±0.01	4.68±0.00
TSC (g/L)	49.65±0.01	42.07±0.16
VSC (g/L)	43.46±0.39	32.24±0.89
Alkalinity (mg/L as CaCO <sub>3</sub> )	56±1	28±0
COD (mg/L)	29219±5666	42553±6137
TKN (mg/L)	5180±0	1245±11
Protein (mg/L)	7583±505	7781±66
TON (mg/L)	92±6	ND
Carbohydrate (mg/L)	602±0	ND
Sulfate (mg/L)	258±1	ND

ND = Not determined.

## 2.5 Analytical methods

The volume of biogas produced was measured using water displacement method. The hydrogen content was measured by gas chromatography (Shimadzu GC 14A equipped with thermal conductivity detector, TCD) fitted with a 1.5 m stainless steel column packed with molecular sieve 58 (80/100 mesh). Argon was used as a carrier gas at a flow rate of 30 mL/min. The temperature of the injection port, oven and detector were 100, 50 and 100°C, respectively. 0.5 mL of sampling gas was injected in triplicate. Volatile fatty acid (VFA) was analyzed by GC-FID (Shimadzu GC 8A). A column capillary packed with nitroterephthalic acid-modified polyethyleneglycol (DB-FFAP) and with a length of 30 m was used. The chromatography was performed using the following program: 100°C for 5 min, 100-250°C with a ramping of 10°C/min, 250°C for 12 min. The detector temperature was set at 300°C. COD, pH, total solid content (TSC), Volatile solid content (VSC), alkalinity, total Kjeldahl nitrogen (TKN), protein content, total organic nitrogen (TON), carbohydrate content and sulfate content were determined in accordance with the procedures described in the Standard Methods (26). However, carbohydrate content in POME has not been determined yet in this preliminary batch experiments to investigate the effect on hydrogen production by adding POME into SLS at different mixing ratio. POME is found previously that it contains carbohydrate at a concentration range of 8-25 g/L, which is the real substrate for hydrogen production by dark fermentation (6, 8, 24, 27). The exact content in POME will be analyzed later in consecutive investigation for nutrients optimization by using the optimum mixing ratio obtained from this study.

## 3. Results and discussion

### 3.1 Hydrogen potential of co-digestion of SLS and POME

Characteristics of raw SLS and raw POME shown in the table 1 have high Total Kjeldahl Nitrogen (TKN)

and low chemical oxygen demand (COD) in SLS, resulting in low C/N ratio about 6. In contrast, POME contains C/N ratio about 34, which is much higher than that of SLS. O-Thong et al. (8) reported the optimum C/N ratio is 74 for biohydrogen production from palm oil mill effluent (POME). Therefore, adding POME into SLS could definitely have more suitable C/N ratio for hydrogen production by using dark fermentation.

The optimum pH for hydrogen production was 5.4-5.7 (6, 8, 27). Skim latex serum and palm oil mill effluent have rather low pH of 4.83 and 4.68, respectively. Under low pH condition, free VFA can cause weak acid inhibition (36). These VFA become more toxic due to an increase of their undissociated fraction. The undissociated VFA can freely cross the cell membrane and then dissociate which lowers internal pH and disrupts homeostasis (37). Therefore low hydrogen production yield obtained was also partly contributed by substrates having low pH. Cumulative hydrogen production under thermophilic condition was obtained from co-digestion of SLS and POME is shown in Fig. 1. The result shows less than one day lag phase of all mixing ratio and hydrogen production rate increased dramatically from 2 to 4 days of fermentation time. The stationary phase had been reached in 4 days of fermentation when using more than 75% of SLS. While the fermentation broth contained less than 75% of SLS, giving more POME composition, the stationary phase occurred after the fourth day of fermentation. Cumulative hydrogen production in this experiment ranged from 10.0±0.1 to 42.8±2.0 mL H<sub>2</sub>. The maximum cumulative hydrogen production (42.8±2.0 mL H<sub>2</sub>) was obtained using co-digestion of SLS and POME with mixing ratio of 65:35 (%v/v) with C/N ratio of about 9. The lowest cumulative hydrogen production (10.0±0.1 mL H<sub>2</sub>) was obtained from fermentation of using only POME, having C/N ratio of about 34. Individual POME was a concentrated substrate with high content of lipid, which could potentially inhibit the fermentation process. In Fig. 2 shows

that hydrogen and total gas ( $H_2$  and  $CO_2$ ) production rate increased slightly when the mixing ratio of POME was increased up to 60 (%v/v) because ammonia and sulfate in SLS were diluted and C/N ratio in the mixtures was increased. Cumulative hydrogen production decreased slightly when the mixing ratio of POME was increased to 40 (%v/v) with cumulative hydrogen production of  $39.7 \pm 1.1$  mL  $H_2$ . Hydrogen and total gas production rate decreased dramatically when the mixing ratio of POME was higher than 40% because anaerobic mixed microflora used in this experiment was not previously acclimated with POME. Moreover, phenol and phenolic compounds, which could pass antibacterial and phytotoxic properties, were reported previously that they are contained at high concentration, 200-1000 mg/L, in POME (38). The lowest cumulative hydrogen production at high substrate concentration indicated inhibition caused by the substrate overload (28). The highest hydrogen production rate was obtained using co-digestion of SLS and POME with mixing ratio of 60:40 (%v/v) with hydrogen production rate of  $22.7 \pm 1.4$  mL  $H_2$ /d, corresponding to biogas production rate with high biogas production rate of  $102.6 \pm 4.6$  mL/d as shown in Fig. 2. The lowest hydrogen production rate was obtained from using fermentation of only POME with hydrogen production rate of  $6.9 \pm 0.1$  mL  $H_2$ /d, corresponding to biogas production rate with the lowest biogas production rate of  $28.2 \pm 0.5$  mL/d. Not only lower hydrogen production rate but also the lower hydrogen content was obtained when using more than 45% of POME as shown in Fig. 3. Due to fermentation mechanism, the different hydrogen content in each mixing ratio changed to the pathways that produce more other gases component such as carbon dioxide. The lowest hydrogen content was obtained using POME with hydrogen content of  $9.7 \pm 0.2\%$ . The hydrogen content increased slightly when increasing the mixing ratio of POME because

ammonia and sulfate in SLS were diluted and C/N ratio in the mixtures was increased. In this experiment the hydrogen content ranged from  $15.1 \pm 1.4$  to  $27.1 \pm 0.8\%$ . The highest hydrogen content ( $27.1 \pm 0.8\%$ ) was obtained using co-digestion of SLS and POME with mixing ratio of 75:25 (%v/v). The hydrogen production yields ranged from  $2.2 \pm 0.1$  to  $23.8 \pm 1.3$  mL  $H_2$ /g-COD<sub>added</sub> as shown in Fig. 4. The highest hydrogen production yield ( $23.8 \pm 1.3$  mL  $H_2$ /g-COD<sub>added</sub>) was obtained using co-substrate with mixing ratio of SLS to POME of 65:35 (%v/v). The hydrogen production yield achieved from co-fermentation was 1 and 5 times higher than that achieved from individual fermentations of SLS and POME, respectively. Comparing with other reports, hydrogen production yields obtained from this study are considerably low. This could be due to several reasons such as no external nutrients adding, no initial pH adjustment, and high initial substrate concentration (30 g-VS/L) and/or microbial toxicants of sulfate and phenolic compounds contained significantly in SLS and POME, respectively. Therefore, further optimizations on those mentioned impacts are needed in order to improve hydrogen productivity from co-fermentation of SLS and POME.

There are mainly four fermentation types in the anaerobic acidogenesis of organic matters (e.g. glucose), namely acetic acid fermentation, propionic acid type fermentation, butyric acid type fermentation, and ethanol type fermentation (29-31). Many microbial communities exhibit acetic acid fermentation with acetic acid as the major product (Reaction (1)) (32, 33). The major products of propionic acid type fermentation are propionic and acetic acids (Reactions (1) and (2)), while the products of butyric acid type fermentation include butyric and acetic acids (Reactions (1) and (3)). As for ethanol type fermentation, ethanol and acetic acid are the primary fermentation products (Reactions (1) and (4)) (30, 31).

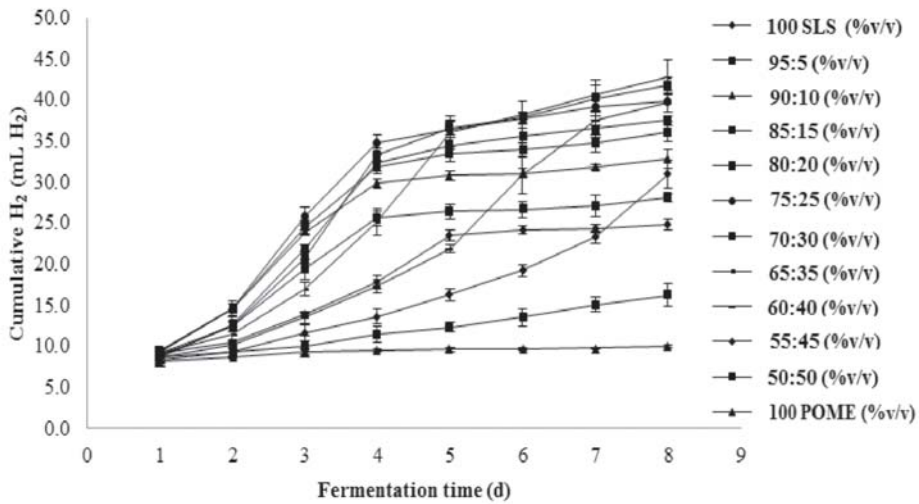


Figure 1. Cumulative hydrogen production at different mixing ratio of SLS to POME.

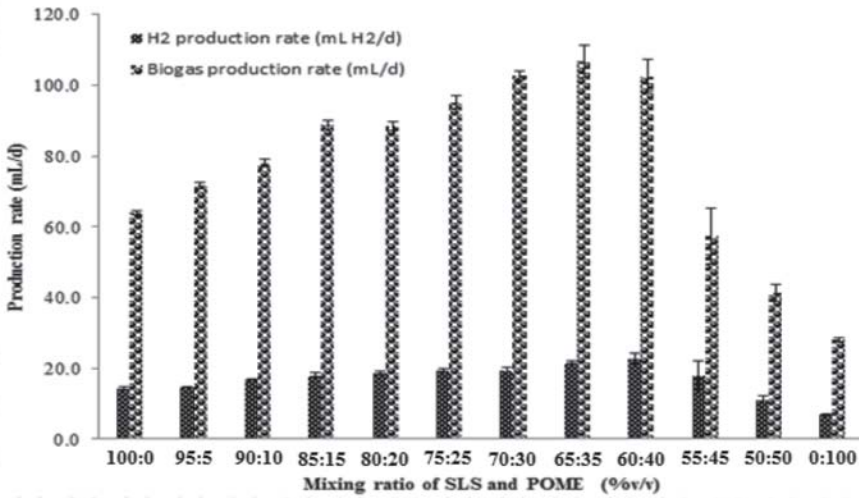


Figure 2. Hydrogen and biogas production rate at different mixing ratio of SLS to POME.

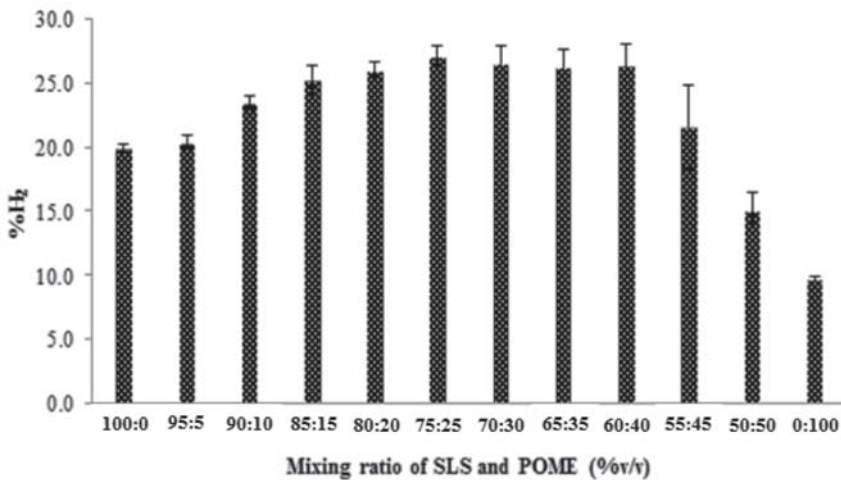
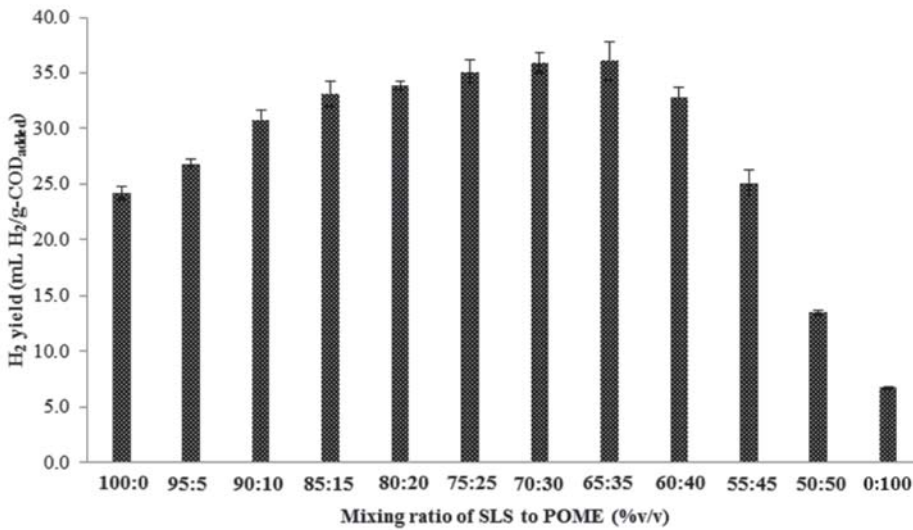
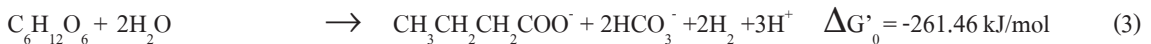
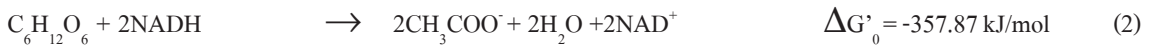


Figure 3. Hydrogen content at different mixing ratio of SLS to POME.



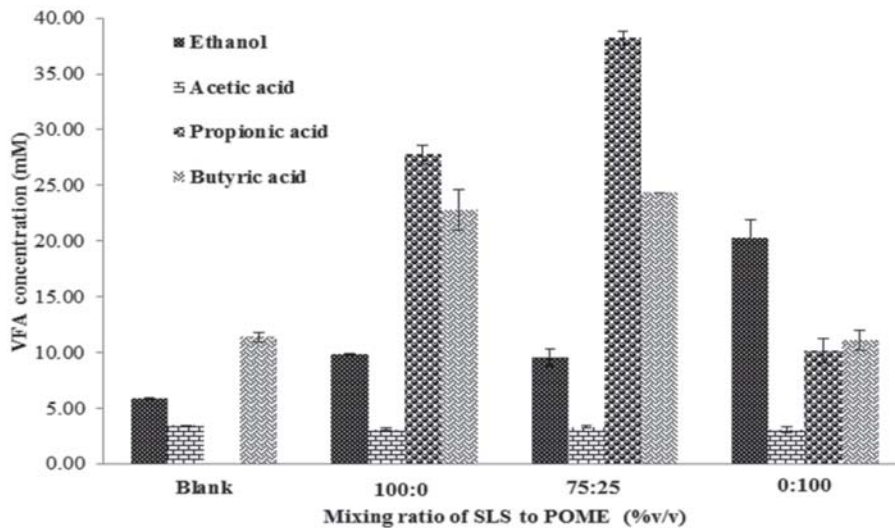
**Figure 4.** Hydrogen production yield at different mixing ratio of SLS to POME.



Reactions (1)-(4) show that hydrogen is generated from acetic acid, butyric acid and ethanol fermentations, not from propionic acid fermentation. However, propionic acid fermentation supposedly occurs much easier than other fermentation types, due to its low Gibbs free-energy change  $\Delta G'_0$  (Reaction (2)) (30, 31). Propionic acid type fermentation is concurrent with other fermentation types which can produce hydrogen (e.g. acetic acid-, butyric acid- and ethanol-type fermentations) in a mixed microbial community. The results of soluble metabolite composition in this experiment are shown in Fig. 5. Propionic acid and butyric acid were the main VFA constituents ( $38.20 \pm 0.63$  mM and  $24.35 \pm 0.00$  mM, respectively) found at mixing ratio of 75:25 (%v/v) of SLS to POME, while small amount of ethanol and acetic acid ( $9.54 \pm 0.80$  mM and  $3.30 \pm 0.11$  mM, respectively) was detected. Although different intermediates were produced in mixed fermentation culture, butyric acid and acetic acid have been reported as abun-

dant liquid products in anaerobic hydrogen production from mixed culture (34). This is likely due to different types of fermentation pathway used by the mixed anaerobic microorganisms. Wang et al. (35) reported the inhibitory effect of added ethanol and acids on glucose degradation efficiency. The results showed that during fermentative hydrogen production by mixed culture, the substrate degradation efficiency in batch tests tended to decrease from 99.0% to 95.7%, 79.9%, 74.5% and 76.5% respectively, with increasing of ethanol, acetic acid, propionic acid and butyric acid concentration from 0 to 300 mM. The inhibitory effects of high ethanol formation on the ability of mixed cultures to degrade substrate during fermentative hydrogen production were smaller than those of high acetic acid, propionic acid and butyric acid formations. When their concentrations increased from 0 to 10 mM, the inhibitory effects of the formation of acetic acid and butyric acid on the ability of mixed cultures to degrade substrate were

similar, but were both larger than that of generating high propionic acid concentration. When the metabolites concentration increased from 50 to 300 mM, the inhibitory effects of high concentration of propionic acid and butyric acid on the ability of mixed cultures to degrade substrate were similar, but were both larger than that of acetic acid. Fig. 5



**Figure 5.** Soluble metabolites obtained at different mixing ratio of SLS to POME.

## 4. Conclusions

The generating biohydrogen from co-digestion of SLS and POME was successfully achieved. Optimum mixing ratio of SLS to POME was 75:25 (%v/v) with hydrogen production yield of  $35.0 \pm 1.2 \text{ mL H}_2/\text{g-COD}_{\text{added}}$ . It can be concluded that POME plays an important role in improving SLS dark fermentation for hydrogen production.

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