Treating Tapioca Starch Industrial Wastewater Using Two-Phase Multi-Staged Up-Flow Anaerobic Sludge Blanket (MS-UASB)

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ABSTRACT

A laboratory-scale, two-phase multi-staged up-flow anaerobic sludge blanket (MS-UASB) treatment system was monitored over time in order to evaluate its treatment efficiency and performance when treating non-diluted industrial tapioca starch wastewater under ambient temperature in Thailand. The system consisted of an acidification (AC) reactor and MS-UASB reactor and was operated for 280 days. The two-phase MS-UASB achieved a maximum organic loading rate (OLR) of 8 kg-COD/m^3/day for the overall system and reached 80.5% of chemical oxygen demand (COD) removal efficiency. Based on the inlet wastewater of each reactor, the AC reactor removed 61.85% of suspended solids and achieved acidification of the wastewater to produce volatile fatty acids at over 50%. Meanwhile, the MS-UASB reactor achieved 74.5% COD removal efficiency. Further analysis found that the increase in soluble extracellular polymeric substances per bound extracellular polymeric substances (S-EPS/B-EPS) was related to the floating sludge phenomenon, which occurred under excess OLR condition.

1. INTRODUCTION

Tapioca (*Manihot esculenta* Crantz), also known as cassava, is a crop cultivated in tropical and sub-tropical regions that can grow in extreme agro-ecologies. The crop can survive infertile land and withstand drought, and its harvests are less affected by diseases. These attributes have made tapioca an important crop, serving as a dietary energy source throughout the world, especially in African and South American regions. While Thailand is one of the major producers of tapioca, the country does not count the crop as a main staple for consumption (Sowcharoensuk, 2020). Accordingly, the majority of tapioca harvested in Thailand is processed into tapioca products, especially tapioca starch, and is bound for export. Tapioca starch is flexible in terms of functional properties and can be used in a wide range of food and non-food industries (Breuninger et al., 2009; Li et al., 2017). Thailand is known as the world's largest exporter of tapioca starch, accounting for as much as 80 percent of world tapioca starch exports (FAO, 2018) and with an annual production volume of 2 million tons (TTSA, 2021; TTDI, 2000).

The tapioca starch production process involves seven major steps: washing the roots, chopping and grinding the plant, extracting and separating the starch, dewatering and separating the protein, drying, and packaging. The process requires a large volume of water, which becomes a massive amount of wastewater. In Thailand, the industry creates an average of 19 m^3 of wastewater per one ton of produced tapioca starch. This wastewater is high in organic content and acidity (Chavalparit and Ongwandee, 2009; Intanoo et al., 2014; Jiraprasertwong et al., 2019; Thepubon et al., 2020), and the discharge of untreated tapioca starch wastewater into the environment can lead to terrible environmental problems. Generally, anaerobic ponds and anaerobic covered lagoon systems are well-known wastewater treatment systems and are considered solutions for various wastewater producing industries, including the tapioca starch industry (Akunna, 2018; Khanal, 2011; Rajbhandari and Annachhatre, 2004).

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However, such systems have low loading rates, require high hydraulic retention times (HRT), and are associated with large land area requirements and high implementation costs. These limitations have led to the development and promotion of various alternatives. An up-flow anaerobic sludge blanket (UASB) reactor is one such treatment system with potential to achieve a high organic loading rate (OLR) with high removal efficiency of chemical oxygen demand (COD) (Annachhatre and Amatya, 2000; Lu et al., 2015; Van Lier et al., 2015). However, failures in long-term operation of UASB reactors while treating tapioca starch wastewater have been observed, including sludge flotation and washout, which have been ascribed to inappropriate operating conditions (Lu et al., 2015; Wang et al., 2018; Wu et al., 2020).

Previous research by Syutsubo et al. (1998) has found that vigorous biogas production during excessive OLR can increase washout of granular sludge in a UASB reactor. In order to solve this problem, biogas production is reduced by installing multiple gas-liquid-solid separators (GLSs) along the height of the UASB reactor, transforming the system into what is known as a multi-staged UASB (MS-UASB). This solution has been applied toward treating various industrial wastewater types, such as lipid and protein, molasses, and high strength vinasses wastewater (Choeisai et al., 2014; Onodera et al., 2011; Tagawa et al., 2002). In addition, a recent study showed that the two-phase UASB, which adds an acidification unit, can enhance stability in treatment performance and greatly improve stability in the morphology of granular sludge in UASB reactors (Wu et al., 2020). This can be attributed to the separation of ideal operating conditions for major microorganisms in the anaerobic treatment process into acidogenesis and methanogenesis. Therefore, research suggests that the combination of an acidification unit and MS-UASB, otherwise known as a two-phase MS-UASB system, may be able to enhance treatment efficiency and stability of granular sludge when treating tapioca starch wastewater.

This study, therefore, initiated testing using a laboratory-scale, two-phase MS-UASB installation, which was operated under ambient temperatures in Northeast Thailand, the nation's major agricultural region for tapioca production and 54.5% of its total agricultural land (KURDI, 2015), to comprehensively evaluate the installation's performance and efficiency in treating high-strength industrial tapioca starch wastewater. The effect of the OLR on system stability was examined by varying HRT to optimize operational conditions. Furthermore, retained sludge properties, including methane producing activity (MPA), sludge concentration, sludge volume index (SVI), and extracellular polymeric substances (EPS), were periodically evaluated in order to assess operating conditions as well as operational stability.

2. METHODOLOGY

2.1 Experimental set-up

A schematic diagram of the laboratory-scale proposed anaerobic treatment system used in this study is shown in Figure 1. The system consisted of an acidification (AC) reactor and an MS-UASB reactor, with working volumes of 8.5 L (10-cm diameter and 100-cm height internally) and 12.7 L (10-cm width, 20-cm length, and 100-cm height), respectively. The MS-UASB reactor was equipped with three GLSs along its height, as illustrated in Figure 1. The AC reactor was initiated without inoculation. The MS-UASB was initiated with 77.7 g-VSS seed sludge. The seed sludge, at a concentration of 6,100 mg-VSS/L, was obtained from an anaerobic cover lagoon treating tapioca starch wastewater located in a Khon Kaen, Thailand tapioca starch factory.

2.2 Characteristics of tapioca starch wastewater

The influent was tapioca starch wastewater (TSW) obtained from the tapioca starch extraction process within Khon Kaen Province, Thailand's tapioca starch industry. The wastewater had a pH of 4.11-4.24; a COD of 13,800-21,400 mg/L, with a biochemical oxygen demand (BOD)/COD ratio of 0.58-0.65; a total suspended solids (SS) value of 2,800-7,340 mg/L, with a volatile suspended solid (VSS) per SS ratio of 0.58-0.69; a total carbohydrates value of 1,080-1,770; and a protein value of 930-1,625 mg/L. Settleable solids were removed from the influent by gravity settling for 1 h, increasing methane production capability by an average of 15.5% with SS concentration removal (Thepubon et al., 2020). Influent pH was adjusted to 7.0 by the addition of sodium bicarbonate ($NaHCO_3$). Neither nutrients nor trace elements were added to adjust influent nutrient composition.

2.3 System operating conditions

The intention of the present study was to enhance the COD removal and biogas production rates in the anaerobic digestion process using the MS-UASB. The system was operated using a semi-continuous feeding pattern controlled by a mechanical electric timer switch. The influent was fed into the system for 15-minute periods along the experimental period. The system's operating conditions are summarized in Table 1. The experimental period was divided into a start-up period (phases 1 and 2) and an acclimation period (phases 3 to 6), operating with diluted wastewater and non-diluted wastewater, respectively.

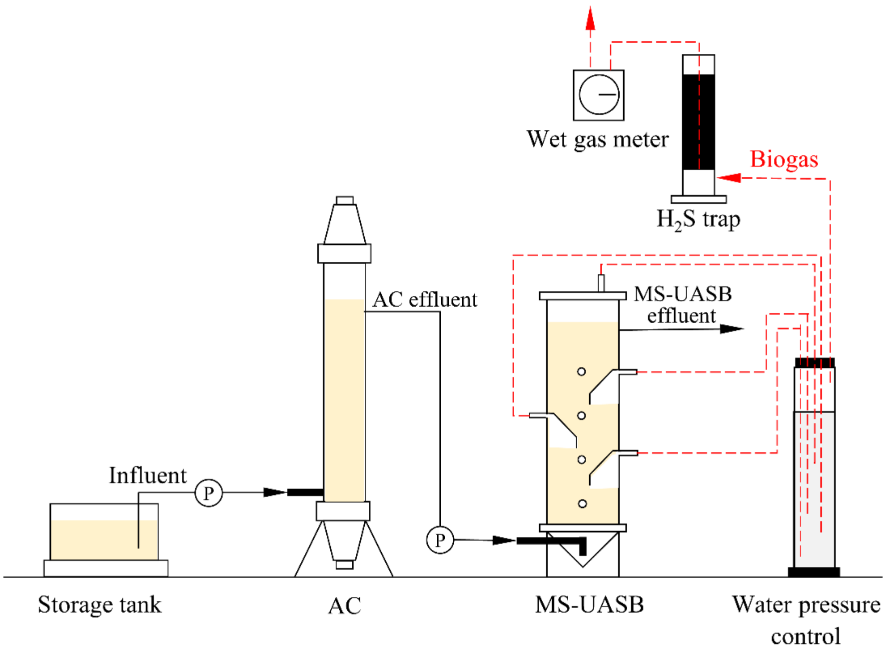


Figure 1. Schematic diagram of laboratory-scale, two-phase MS-UASB system

Table 1. Operating conditions of the two-phase MS-UASB system

Phase	Operation period (day)	Influent COD (mg/L)	AC		MS-UASB	
			HRT (h)	OLR*	HRT (h)	OLR*
1	1-35	2,910.5±388.0	68	1.0±0.1	108	0.5±0.1
2	36-110	5,861.2±498.4	68	2.1±0.2	108	0.8±0.1
3	111-152	15,711.5±2488.6	38	9.8±1.6	58	4.5±0.8
4	153-210	15,116.6±1,692.6	21	17.7±2.7	30	9.1±1.3
5	211-223	10,972.8±1,556.6	10	33.6±1.2	16	17.1±2.4
6	224-280	11,872.1±1,627.8	21	19.8±3.0	30	10.5±1.8

*OLR is given in units of kg-COD/m^3/day

2.4 Analytical methods

The room temperature and internal liquid temperature at mid-height of both the AC and MS-UASB reactors were measured daily. Wastewater samples, including influent, AC effluent, and MS-UASB effluent, were collected to measure COD, SS, and VSS following standard methods (Baird et al., 2017), and volatile fatty acids (VFAs) were measured by direct titration method (Dilallo and Albertson, 1961), in order to monitor the efficiency and performance of the treatment system. In addition, the samples were measured twice for carbohydrate and protein content at each operational phase when COD removal of both reactors was in a steady state. Protein and carbohydrate content were evaluated by Lowry's method (Lowry, 1951) using albumin as a reference solution and phenol-sulfuric acid method (Nielsen, 2017) using glucose as a reference solution, respectively. Biogas production was measured daily by a wet gas meter (Tokyo Shinagawa, model WS-1A), and biogas composition was analyzed using gas chromatography (Shimadzu, GC-8A) with a thermal conductivity detector (TCD).

2.5 Analysis of retained sludge behavior

Retained sludge samples were periodically collected from the 0.15-m high MS-UASB reactor to investigate physicochemical sludge properties, including mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), and SVI. Moreover, each sludge sample was measured for EPS, including bound EPS (B-EPS) and soluble EPS (S-EPS) forms. EPS were extracted using formaldehyde-sodium hydroxide (Liu and Fang, 2002; Chabalina et al., 2008), after which variations in carbohydrate and protein content in both forms of extracted EPS were determined.

In order to investigate methane producing capability, retained sludge samples were collected on Day 0 (seed sludge), Day 132, and Day 223 and were evaluate for MPA. Serum vials were used following the method described by Harada et al. (1994) and Syutsubo et al. (2001), under mesophilic conditions (35±1ºC) at a food/microorganism (F/M) ratio of 0.4 mg-COD/mg-VSS. The substrates used in MPA testing included acetate, H_2/CO_2 (80/20, v/v), and tapioca starch wastewater.

3. RESULTS AND DISCUSSION

3.1 Performance of two-phase MS-UASB system

The two-phase MS-UASB system was operated for 280 days, divided into the six phases described in Table 1, under ambient temperatures. Figure 2 shows the system's operating conditions and performance over time along the experimental period. Ambient and liquid temperatures inside both reactors remained between 20-33ºC and averaged 28±2.4ºC (as shown in Figure 2(a)). During the start-up period, in phases 1 and 2, the COD removal efficiency of the overall system was maintained above 80%. After successful start-up, non-diluted TSW was fed to the system in phases 3-6. Phases 3-5 could be considered an acclimation period and phase 6 a recovery period. During the acclimation period, OLR was increased by decreasing HRT. Phase 4 was the most stable period, with the overall system reaching its highest OLR of up to approximately 7 kg-COD/m^3/day (18 kg-COD/m^3/day at the AC reactor and 9 kg-COD/m^3/day at the MS-UASB reactor), and an average total COD (T-COD) removal efficiency of 80.5% (22.3% at the AC reactor and 74.5% at the MS-UASB reactor). However, sludge flotation and washout occurred in the MS-UASB in phase 5 on Day 223, which was 12 days after operation had been characterized by an HRT of 26 h and OLR of 14 kg-COD/m^3/day (33 kg-COD/m^3/day at the AC reactor and 17 kg-COD/m^3/day at the MS-UASB reactor).

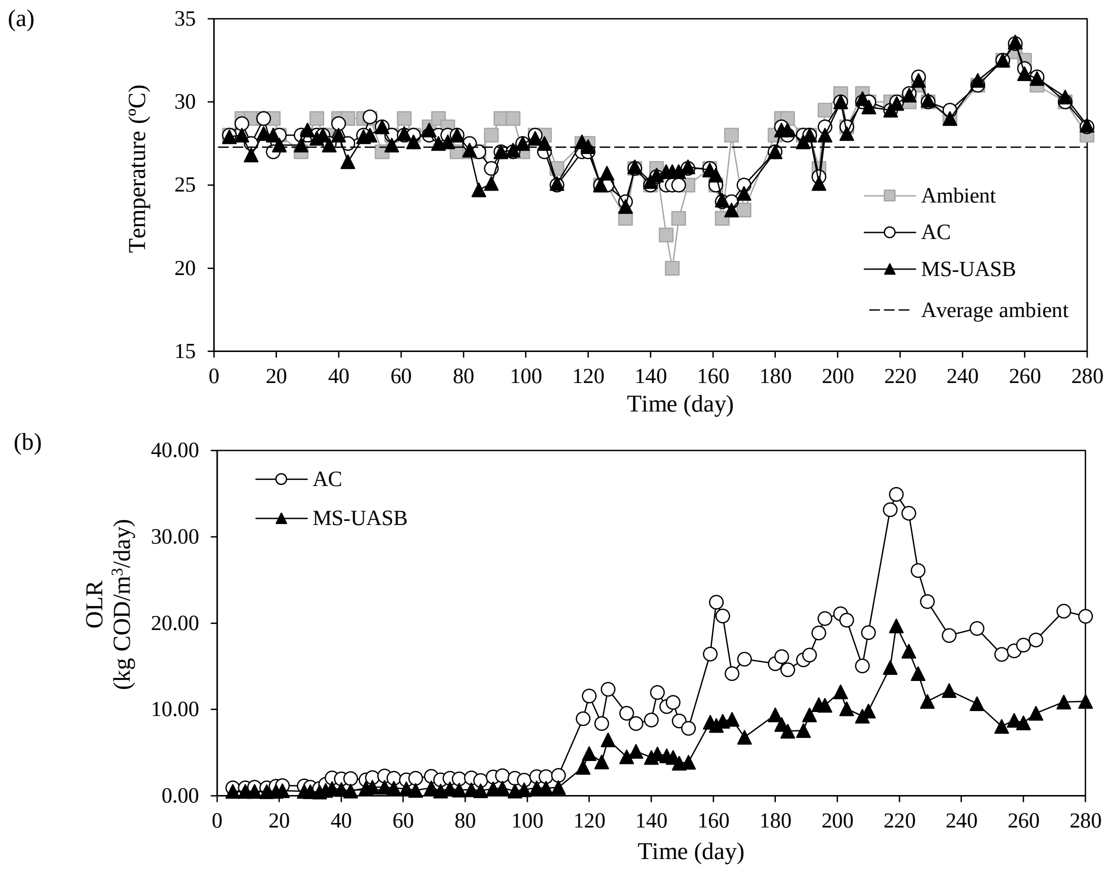


Figure 2. Operational performance of two-phase MS-UASB system during experimental period

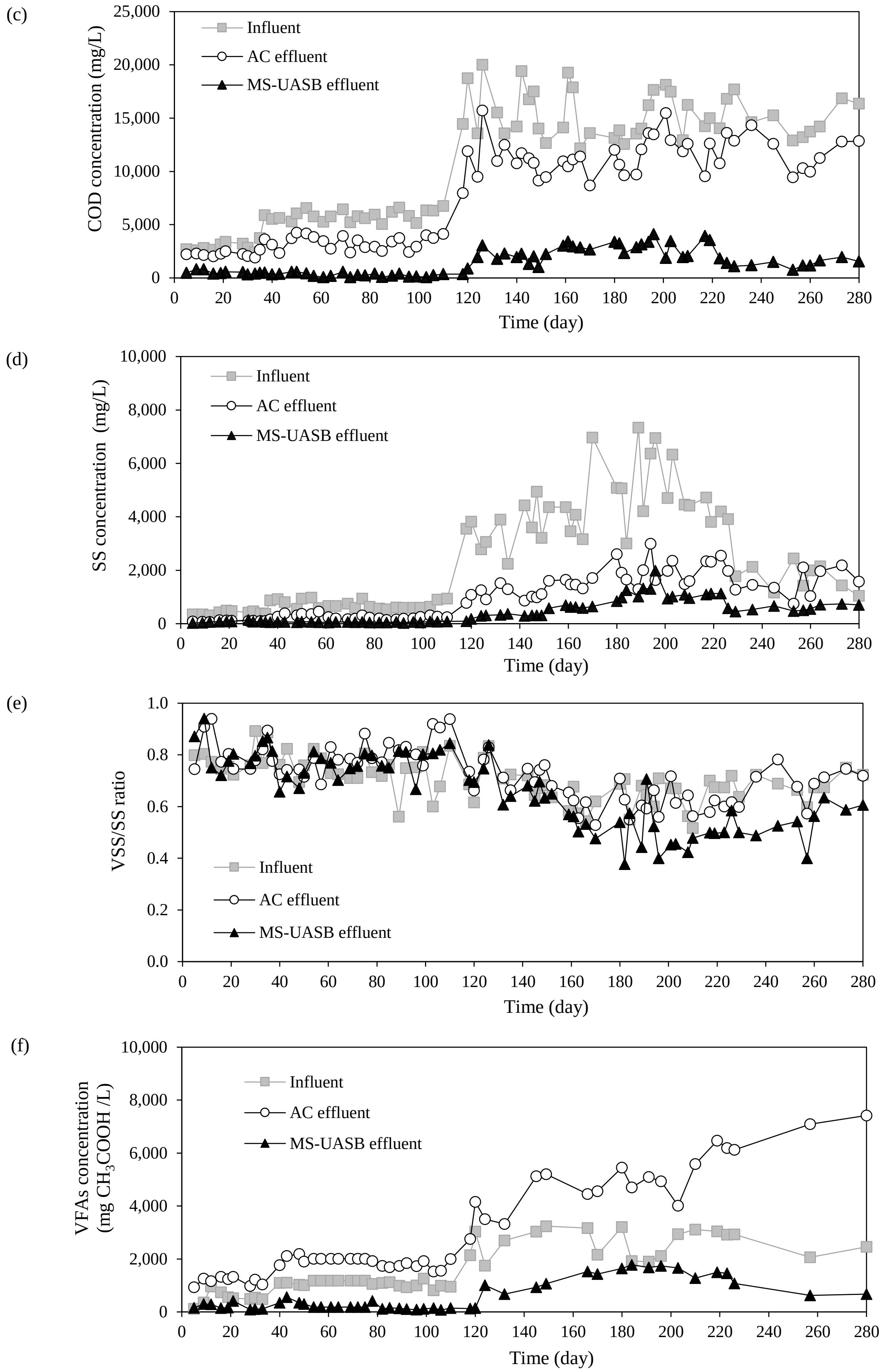


Figure 2. Operational performance of two-phase MS-UASB system during experimental period (cont.)

(g)

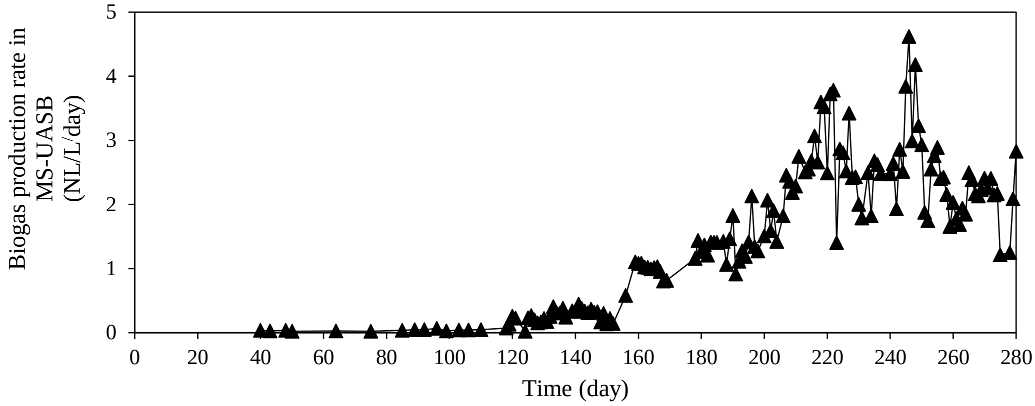


Figure 2. Operational performance of two-phase MS-UASB system during experimental period (cont.)

In order to solve this problem, the HRT was immediately extended to 51 h as in phase 4. The HRT remained at 51 h throughout phase 6, or the recovery period, from Days 224-280. In the recovery period, the average T-COD removal efficiency of the AC reactor was 20.9%, while that of the MS-UASB increased to 88.8% based on inlet COD, indicating a higher efficiency than that measured under similar HRT conditions in phase 4, during the acclimation period. The increase of COD removal efficiency was attributed to an increase in the average ambient temperature by approximately 3ºC as a result of seasonal weather changes in Thailand, as shown in Figure 2 (a). It could be concluded that the two-phase MS-UASB for treating high-strength industrial TSW was able to achieve a maximum available OLR of 8 kg-COD/m^3/day with an HRT of 51 h for the overall system. Based on inlet wastewater for each reactor, the AC reactor and the MS-UASB reactor achieved an OLR of 19 kg-COD/m^3/day and 9 kg-COD/m^3/day, respectively.

During phase 4, the AC reactor achieved SS removal at an average of 61.8±10.1% and the VSS/SS ratio of the AC reactor influent and effluent showed similar characteristics, averaging 0.62±0.06 and 0.61±0.06, respectively. Differences in average T-COD and soluble COD of the AC reactor's influent and effluent (data not shown) revealed that the COD caused by suspended solids (SS-COD) in the influent and effluent was at 23.9% and 12.3%, based on each T-COD, respectively. SS-COD removal at the AC reactor was 56.2% based on SS-COD influent, or 16.6% based on T-COD influent (T-COD removal at the AC reactor was 22.3% based on T-COD influent). Consequently, the AC reactor trapped more than 50% of the influent SS without changing VSS/SS ratio characteristics, and 74.4% of T-COD removal was COD removal from the trapped SS fraction. The MS-UASB reactor, on the other hand, achieved SS removal at an average of 43.5%. The VSS/SS ratio of the MS-UASB effluent changed to 0.50±0.08, a 10% decrease from the AC reactor effluent. This indicated that the MS-UASB reactor achieved removal of the organic fraction of SS particulate matter to a modest degree.

In terms of VFAs concentration (Figure 2(f)), that of the AC reactor effluent increased by more than 50% throughout the experimental period, indicating that an acidogenesis reaction occurred in the AC reactor. Subsequently, the MS-UASB reactor, fed with AC reactor effluent, decreased the VFAs concentration by up to 60%. VFAs concentration of the MS-UASB effluent in phase 4 averaged 1,600 mg/L as acetic acid, and VFAs effluent concentration was stabilized.

The methane content in the biogas depended on a variety of factors, including the particulate matter in the wastewater, the pH in the reactor, and nutrient limitations. The methane potential of each wastewater sample needed to be evaluated using small scale batch reactor performance test results (Angelidaki and Sanders, 2004). In phase 4, the MS-UASB influent, or the AC-reactor effluent, had a high SS concentration, averaging 1,820±475 mg SS/L, the pH of the effluent averaged 7.94±0.07, and the methane content (as shown in Figure 2(g)) was measured and averaged at 76.5±10.2%. However, it should be noted that fluctuations in biogas production (data not shown) occurred within the MS-UASB reactor, and the methane potential was calculated to be 0.69±0.28 NL-CH_4/L-TSW, equivalent to a methane yield of 0.27±0.16 NL-CH_4/g-$COD_{removed}$.

The aforementioned two-phase MS-UASB performance results indicate that the installation of an AC reactor as a pre-treatment unit enhanced treatment performance and reduced the risk of inhibition within the MS-UASB reactor. Clearly, the use of a two-phase

MS-UASB could help to reduce retention time from approximately four days of degradation duration time as measured by the biochemical methane potential test to two days for achieving maximum biodegradation potential (Thepubon et al., 2020).

3.2 Biodegradation of macro-pollutants in TSW

As shown in Figure 3(a) and 3(b), carbohydrate and protein, the major macro-pollutants in starch wastewater (Devereux et al., 2011), were measured under all operating conditions throughout the experimental period. The AC reactor achieved a carbohydrate removal efficiency of over 80% under all operating conditions while protein removal levels fluctuated. Protein removal efficiency, varying between 30-67%, mainly occurred at the MS-UASB reactor. These results clearly indicate that carbohydrates were effectively converted to acetic acids and other VFAs during the acidification that occurred at the AC reactor, similar to findings from previous research (Wu et al., 2020). In contrast, the protein was largely hydrolyzed and acidified under methanogenesis conditions, consistent with research by Miron et al. (2000). In addition, protein was a macro-pollutant requiring a longer HRT, with a lower biodegradation rate than carbohydrates (Akunna, 2018; Cremonez et al., 2021). This suggests that optimal conditions for the acidification unit should be further investigated in order to enhance the performance of the two-phase MS-UASB when treating tapioca starch wastewater.

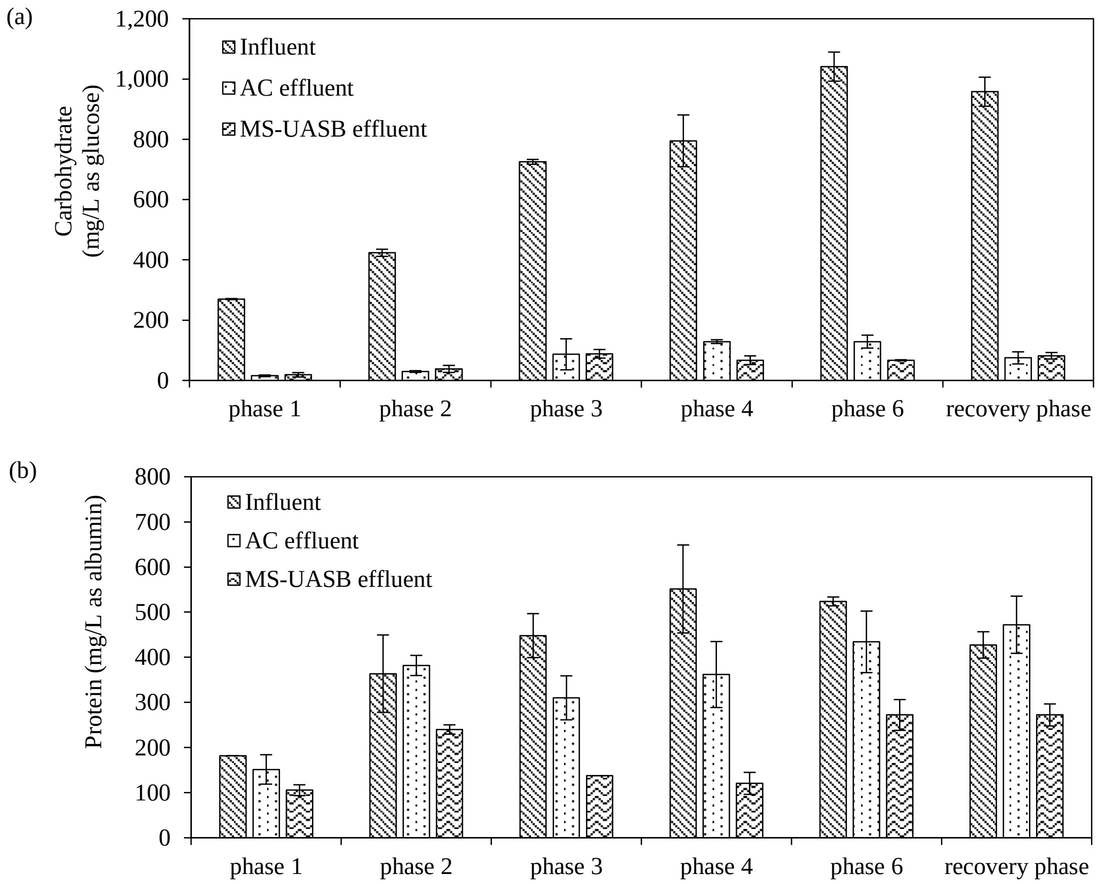


Figure 3. Treatment performance of the system in terms of (a) carbohydrate and (b) protein components

3.3 Methanogenic producing activities (MPA) of the retained sludge

Methanogenesis in anaerobic digestion stages is generally separated into *Acetoclastic methanogen* and *Hydrogenophilic methanogen* (Akunna, 2018). *Acetoclastic methanogen* uses acetic acid to produce methane, with 72% of methane produced from *Acetoclastic methanogen*, and the remaining 28% of methane produced from *Hydrogenophilic methanogen* by utilizing H_2 and CO_2 (Khanal, 2011). In the MPA test conducted for the present study, acetate and H_2/CO_2 (80/20) were used as test substrates to uncover the activity of these two types of methanogens, and TSW was used as a test substrate to evaluate the MPA of the retained sludge from the TSW. The changes in MPA of the retained sludge are shown in Figure 4. The sludge samples included seed sludge (day 0), retained sludge from the acclimation period in phase 3 (Day 132), and retained sludge from the first day that sludge flotation occurred in phase 5 (Day 223). The results showed that when using acetate as the test substrate, the MPA of the retained sludge had increased 2.1 times by Day 132 and 4.6 times by Day 223 compared to that of the seed sludge; when using H_2/CO_2 as the test substrate, changes in MPA in the retained sludge were insignificant when compared to that of the seed sludge; when TSW was used as the test substrate, the MPA of the retained sludge was shown to have increase 1.6 times by Day 132 and 5.9 times by Day 223 compared to that of the seed sludge. These results indicate that the TSW was acclimated in the MS-UASB, and the two-phase MS-UASB did separate between the acidogenesis and methanogenesis reactions under ex-situ conditions. Consequently, MPA was shown to significantly increase when acetate was used as the source for methane production. This is typical degradation that occurs due to *Acetoclastic methanogen*.

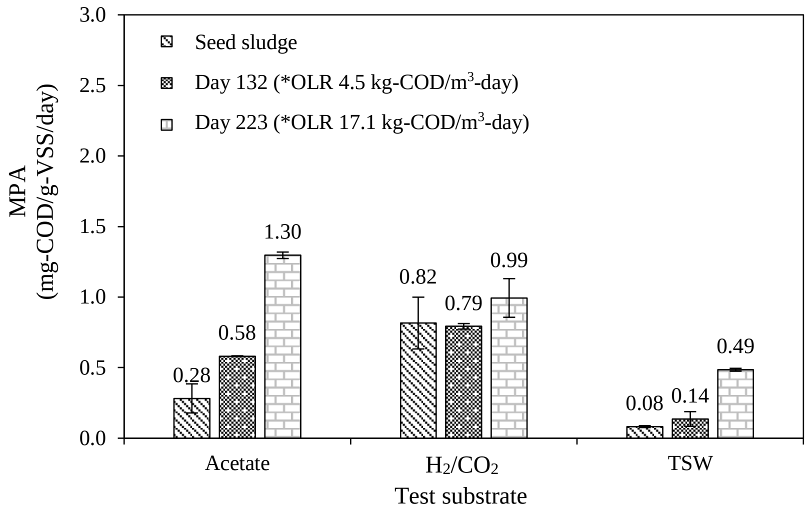


Figure 4. MPA of seed sludge and retained sludge sampled on Days 132 and 223 (OLR*: the average OLR of all phases of the MS-UASB reactor)

3.4 Physicochemical properties of retained sludge

Aside from the operational conditions, physical and chemical properties of the retained sludge in terms of MLVSS concentration, SVI, B-EPS, and S-EPS were analyzed and are shown in Figure 5. Sludge properties can influence performance and stability of anaerobic wastewater treatment systems (Wang et al., 2018). Higher MLVSS concentrations (ranging from 30,000-50,000 mg/L) and lower SVI values (<20 mL/g MLVSS) are preferable for obtaining good granulation of UASB retained sludge (Cervantes et al., 2006). As shown in Figure 5(a), during the acclimation period, the MLVSS concentration of the MS-UASB retained sludge increased and stabilized at 9,300 mg/L, with a low SVI of 17.7 mL/g-MLVSS. This was better than the MLVSS concentration of the seed sludge, which was 6,120 mg/L, with an SVI of 40.4 mL/g-VSS. These results confirm these advantageous properties of MS-UASB retained sludge.

Moreover, TSW's SS concentration of over 500 mg/L is considered high for a UASB system, as SS adsorption has been evidenced to affect granular sludge properties (Stronach et al., 2012; Van Lier et al., 2015). EPS biosynthesis, achieved either by microorganism secretion or adsorption of certain organic compounds, significantly influences sludge properties (Sheng et al., 2010). Consequently, retained sludge and floating sludge were evaluated for EPS along the experimental period. EPS is separated into two forms: B-EPS and S-EPS. B-EPS is described as an inner layer tightly and closely bound with cells, while S-EPS is weakly bound with cells dispersed in the solution. Normally, microbial aggregates contain higher B-EPS content than S-EPS content. Both forms of EPS are comprised largely of polysaccharides (carbohydrates), with proteins as the major substance. Previous studies have focused on the ratio of protein/carbohydrate as a parameter relating to sludge characteristics (Lu et al., 2015; Wu et al., 2020; Liu et al., 2010; Lu et al., 2018; Wang et al., 2018). However, the present study found protein to be a major chemical substance that significantly changed the concentration of both forms of EPS throughout the experimental period. This resulted in the retained sludge having a protein/carbohydrate ratio in line with EPS content, which may be attributed to the adsorption of protein compounds. These protein compounds were a major macro-pollutant fed into the MS-UASB reactor, as mentioned in section 3.2. Consequently, the protein/carbohydrate ratio of EPS content was not a focus in the present study; rather, S-EPS/B-EPS was considered a useful parameter relating to the retained sludge properties in this study.

As shown in Figure 5(b), total B-EPS content did not change significantly in the start-up period. Total S-EPS content, however, decreased dramatically within 32 days after the start-up period (-8.8 times from seed sludge). The S-EPS/B-EPS ratio of retained sludge on Day 32 was 0.08, an 87.5% decrease from the seed sludge, which contained an S-EPS/B-EPS ratio of 0.62. The B-EPS content of retained sludge on Days 132, 223, and 246 then increased 1.8, 3.2, and 4.4 times, respectively, relative to the retained sludge on Day 32, while the S-EPS content increased 1.5 times, 3.6 times, and 4.5 times, respectively. During this period, the S-EPS/B-EPS ratio of retained sludge remained in the range of 0.06-0.08.

In addition, a floating sludge phenomenon occurred on Day 223. The floating sludge was collected and analyzed for both forms of EPS content, then compared to the retained sludge on the same experimental day, the results of which are shown in Figure 5(c). The comparison showed that the total B-EPS content of the floating sludge was 48.1% lower than that of the retained sludge, while S-EPS content was 98.9% higher than that of the retained sludge. The S-EPS/B-EPS ratio of the floating sludge was 0.31, 3.9 times higher than that of the retained sludge.

Based on the findings described above, the decrease in S-EPS and the lower S-EPS/B-EPS ratio in the retained sludge on Day 32, relative to the seed sludge, indicated an elimination of the poor settleable sludge containing a high S-EPS/B-EPS ratio. In other words, a selection and aggregation of retained sludge occurred in this period, increasing biomass concentration and improving settleability. Then, the S-EPS/B-EPS ratio of the retained sludge during the acclimation and recovery period was maintained in the range of 0.06-0.08, and biomass concentrations and SVI remained stable. The floating sludge on Day 223, on the other hand, was shown to have an S-EPS/B-EPS ratio of 0.31, suggesting that a higher S-EPS/B-EPS ratio may indicate an increase in the production of weakly bound EPS on the sludge surface, resulting in poor settleability and sludge flotation. Meanwhile, the retained sludge with the lower S-EPS/B-EPS ratio would have a more stable structure, resulting in high settleability with low SVI.

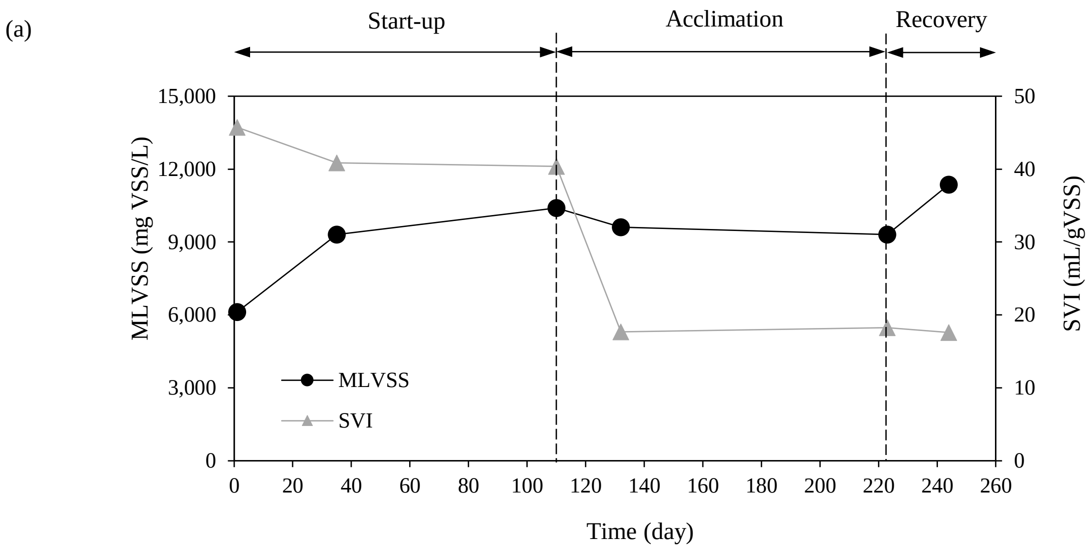
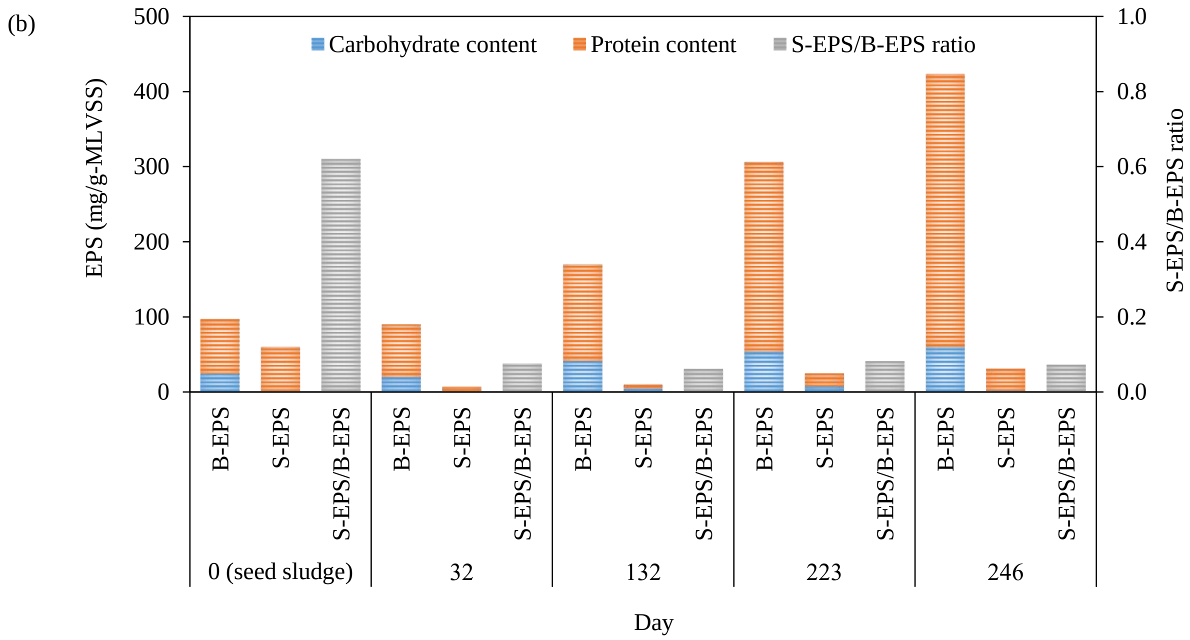


Figure 5. Physicochemical properties of sampled MS-UASB sludge over time, including (a) MLVSS and SVI, (b) EPS, and (c) comparison between EPS of retained sludge and floating sludge on Day 223



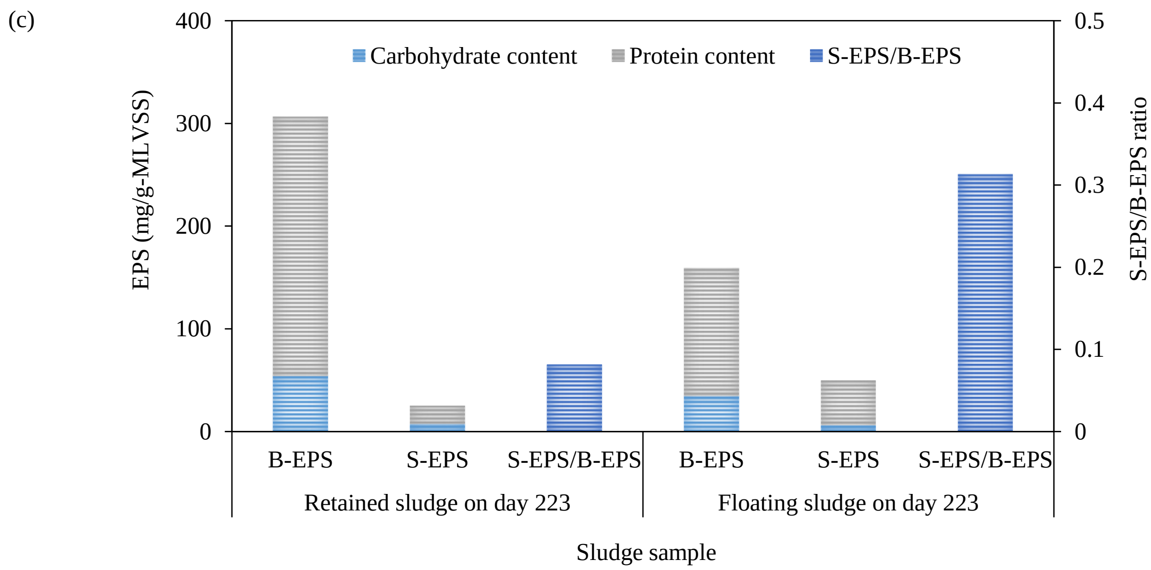


Figure 5. Physicochemical properties of sampled MS-UASB sludge over time, including (a) MLVSS and SVI, (b) EPS, and (c) comparison between EPS of retained sludge and floating sludge on Day 223 (cont.)

4. CONCLUSION

Treatment of non-diluted TSW by the two-phase MS-UASB system provided a stable performance, with the overall system averaging 80.5% COD removal under a maximum available OLR of 8 kg-COD/m^3/day and at ambient temperatures of 28.0±2.4°C. The AC reactor removed 61.8% of SS and degraded over 80% of carbohydrates into VFAs. The MPA of the MS-UASB retained sludge on Day 224 was at 1.3 g-COD/g-VSS/day using the acetate test substrate, with a high MLVSS concentration of 9,300 mg/L, a high settleability with low SVI at 17 mL/g-MLVSS, and a low S-EPS/B-EPS ratio of 0.08. The MS-UASB system achieved a methane yield of 0.27 NL-CH_4/g-$COD_{removed}$.

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