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Effect of pH on Acidification of Whey, Yeast and Yolk Wastewaters

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ABSTRACT

The acidification of protein wastewaters was investigated under different operational pH values (4.14 – 7.09) at ambient temperature. Synthetic whey, yeast and yolk wastewaters were used as the substrates. Three lab-scale continuously stirred tank reactors (CSTRs), each with a working volume of 2 L, were employed as acidogenic reactors. They were operated at a hydraulic retention time (HRT) of 4 h with a substrate concentration around 2 gCOD/L, corresponding to organic loading rates (OLRs) of 12.9 – 14.7 gCOD/L· d. Whey wastewater was the most sensitive to pH with the maximum acidification efficiency of 30.9% and acidification rate of 3.88 gCOD/L· d at pH 4.14. The highest acidification efficiency and acidification rate of yeast wastewater were found in neutral pH range (7.09) at 4.6% and 0.52 gCOD/L· d, respectively. Yolk wastewater was better acidified in slightly acidic condition (pH 5.52) with the maximum efficiency and rate at 4.6% and 0.38 gCOD/L· d, respectively. When comparing the system performance with no pH control condition of each wastewater, pH adjustment is not necessary. The results of this study confirmed that pH adjustment cost could be saved in the acidogenic CSTR of the two-phase anaerobic treatment of the protein wastewaters.

Keywords: Acidification efficiency, Acidification rate, CSTR, pH, Whey, Yeast, Yolk

1. Introduction

The imbalances of acid-forming bacteria and methane-formatting bacteria caused the accumulation of intermediary acid products which provided the inhibition of methanogenic bacteria. This rationale led to the separation of the acidogenic and methanogenic phase into two-phase systems [1-2]. The anaerobic two-phase reactor needs a significant amount of a high total chemical oxygen demand (TCOD) content in the influent substrate for the fermentation process. The acidification converts organic matter in the influent wastewater into volatile fatty acids (VFAs) by acidogenic microorganisms. VFAs are the intermediate products from the fermentation process which were further converted to methane in the successive methanogenic reactor [3-4].

Acidification process is mainly influenced by a series of operational parameters, such as HRT, OLR, pH and temperature [5]. The pH affected the bacterial growth rate while pH changes may cause a drastic variation in the relative number of different species in the heterogeneous population in the acidogenic reactor [6]. Many studies found the influence of pH on the acidogenic process inferring that a slightly acidic pH near 6.00 improved the reactor performance for acidogenesis bacterium [7-8]. Wang et al., [9] found that high VFA production could be obtained by fermenting food waste seeded with anaerobic sludge at pH 6.00.

The process of anaerobic digestion undergoing a high OLR could be tended to deterioration even failure due to direct inhibition and VFAs overload. More often, process instability could be affected by rapid changes in feedstock composition or OLR and unsuitable pH range [10-11]. Han et al. [12] operated multistep sequential batch two-phase anaerobic treatment for the food waste and found a suitable pH range between 6.50 – 7.00 during the acidogenic step. The favourable pH conditions in acidogenesis were 5.20 – 6.50, while they were 6.60 – 8.50 for acetogenic/methanogenic bacteria in dairy manure treatment [13]. Nevertheless, the information on the effect of the acidification

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process under various pHs is rather limited in protein wastewater.

Many studies have been carried out on acidification of wastewater such as dairy, cheese whey, starch, lactose and gelatin. A few full-scale reactors have been applied to treat wastewater [7], [14 – 19]. The information on acidification of protein wastewater is rather limited although many industrial and agricultural wastewaters contain appreciable quantities of protein. Protein is degraded slower than carbohydrate but faster than fat. Type of protein in wastewater, HRT, substrate concentration and pH were essential factors which affected the acidogenic process. The slowly biodegradable matters were found in the protein and fat-containing wastewaters which decreased the performance of the reactor [20]. Treating protein - rich wastewater often results in the formation of scum which accumulates inside the reactor and causes sludge to wash out [21].

Proteins are composed of carbon, nitrogen, and oxygen. Each protein had a polypeptide backbone and associated with each peptide. In which a peptide and its associated side group are called an amino acid. There are 20 amino acids, and the variations in lengths and arrangement determine the different protein. The type of acidogenic fermentation was different for each kind of protein, so it was probable that the conversion pathways to VFAs from proteins would be different. Soluble protein can be hydrolyzed to amino acid then to NH_4^+-N, pyruvates, and other products. Pyruvates can be transformed directly into VFAs or lactate, which can also be converted to VFAs [22]. Three types of synthetic protein wastewaters which generally found in industrial processing were investigated in this study, i.e., whey (dairy, cheese, bakery), yeast (beer, fermentation process), yolk (animal feed, bakery, ice cream). The objective of this study was to investigate the effect of pH on the acidogenesis of the above mentioned three types of protein wastewaters in CSTRs under ambient temperature.

2. Material and methods

Synthetic protein wastewater was daily prepared from a mixture of tap water (90% by volume), protein substrates for carbon source and domestic wastewater from Chiang Mai University (CMU) wastewater treatment plant (10% by volume) for micronutrients and trace elements. The domestic wastewater was weekly collected from equalizing tank and stored in 4 °C room before utilization. Tap water was stored overnight to reduce residual chlorine. Three types of protein substrates, i.e., whey powder (Cottage farm ®, USA), yeast extract (Marmite ®, UK) and yolk powder (Yok Intertrade, Thailand) were mixed with tap water and domestic wastewater to the required Chemical Oxygen Demand (COD) concentrations. Total Kjeldahl nitrogen (TKN) and total phosphorus in synthetic wastewaters were analyzed and the COD: N: P ratios were 100: 2: 1.2, 100: 6: 1.8 and 100: 4: 1.2 for whey, yeast and yolk wastewaters, respectively. It indicated a sufficient macronutrient ratio for the bacterial grow at 100: 2: 0.2 [3]. The characteristics of protein substrates were analyzed by the Bureau of Nutrition, Department of Health, Thailand as presented in **Table 1**.

Table 1: The characteristics of protein substrates based on 100 g of wet weight (n = 3)

Parameter	Unit	Type of substrates		
		Whey	**Yeast**	**Yolk**
Carbohydrate	g	75.9	25	2.3
Protein	g	10.6	39	29.9
Fat	g	1.9	< 1	61
Water	g	4.8	31.9	3.7
Other ash	g	6.8	3.1	3.1

The digested sludge from an anaerobic sludge digester of CMU wastewater treatment plant, (20.5 gVSS/L) was used as the seed sludge in CSTRs. The seed volume (1.08 – 1.25 L) was mixed with protein wastewaters at ambient temperature for two weeks before loading. Three CSTRs, made from 15.24 cm PVC pipe with 20 cm height which giving a working volume of 2 L, were used. The influent stored in a 20 L plastic tank equipped with a circulating pump was introduced into each CSTRs by a metering pump. A centrifugal pump was used to circulate the liquid content in a CSTR to provide the complete- mix condition. The effluent flowed from each reactor to a 20 L plastic effluent tank. Each CSTR was coupled with 350 mL water replacement plastic bottle submerged in 500 mL beaker to collect the biogas. After the system reached the steady state, i.e. effluent VFA fluctuations within 15%, data during steady state conditions were investigated.

The experiment was conducted from February to July 2016. The CSTRs were started up at 24 h HRT before stepwise decreased to 20, 16, 12, 8 and eventually 4 h which was maintained throughout the study. Three parallel experiments for whey, yeast and yolk wastewaters were operated under the influent substrate concentration of 2 gCOD/L. The influent in each reactor was adjusted by 4 N HCl, 4 N NaOH or 4 N $NaHCO_3$ to the required pH condition. The reactor's pH (similar to effluent pH) in each CSTR was adjusted as follows: reactor 1 (whey wastewater) 4.18, 5.49 and 6.50; reactor 2 (yeast wastewater) 4.50, 5.57 and 7.09; reactor 3 (yolk wastewater) 4.55, 5.52 and 6.56. For comparison, data from the similar experiment of three wastewaters without pH control at 4 h HRT during January – February 2016 are included. They are parts of the study on the effect of HRT on acidification without pH control and results are presented elsewhere [23].

The influent and effluent samples were collected from the influent and effluent tanks, respectively. All essential parameters, i.e., COD, pH, suspended solids (SS), volatile suspended solids (VSS) and VFA were analyzed according to Standard Methods [24]. VFA compositions (acetate, propionate, butyrate, *i*-butyrate, valerate, and *i*-valerate) were also analyzed as follows; firstly, a 5 mL of the filtered sample was acidified with 4 N phosphoric acid to a pH less than 3 to convert the fatty acids to their undissociated forms (i.e., acetic acid, propionic acid, butyric acid, etc.). A 1 μL of the acidified sample was analyzed for acetic acid, propionic acid, i- and n-butyric acid, i- and n-valerate by Agilent 680 Gas Chromatograph (GC) with HP – FFAP column of 25 mm × 0.32 mm × 0.5 μm and a Flame Ionization Detector (FID). The temperature of the column, injection, and detector were 80°C, 200°C and 250°C, respectively.

To assess the acidification efficiency (AE) and volumetric acidification rate (AR), a mass balance on COD of influent and effluent VFA concentration and VFA converted to biogas were done at steady state condition using Equation 1 - 3 [3, 25] as follows:

$$COD_{acid} = \text{effluent}COD_{VFA} - \text{influent}COD_{VFA} + COD_{CH4} \quad (1)$$

$$AE = \frac{CODacid}{\text{influentCODnon}-VFA} \times 100 \quad (2)$$

$$AR = \frac{CODacid \times \text{flow rate}}{\text{Volume of reactor}} \quad (3)$$

COD_{VFA} is the fraction of COD presented as VFAs (mgHAc/L) and calculated from theoretical COD equivalent of 1.066 gCOD/gVFA. CODnon-$_{VFA}$ is the total COD minus COD_{VFA}. COD_{CH4} is the fraction of COD converted into methane gas in which it was neglected in this study due to very low and no methane production at 4 h HRT.

The overall performances of the system under the varied operational conditions were evaluated for the statistical significance analysis by using Minitab®. The significant test as p-value was examined at a confidence level of 95% [26].

3. Results and discussion

The results of the study including data without pH control [23] are summarised in **Table 2**. The acidogenic CSTRs were operated at 4 h HRT, corresponding to OLRs 12.9 – 14.7 gCOD/L· d. The substrate composition presented in **Table 1**, resulted in various influent characteristics. Yolk powder had a much higher SS and VSS values while whey wastewater gave the distinctly highest VFA concentration. Although the target influent COD was 2 g/L, deviations during the experiment led to slightly different values. The influent temperatures were in the range of 25.1 – 35.2℃. The effluent temperature which was the same as the reactor's temperature was measured instantly in the effluent pipe and found to be lower than the influent and be within mesophilic range (20 - 40℃). Due to the seasonal change in Chiang Mai, Thailand, the ambient temperature usually dropped during winter (November to February). The effect of temperature at the study range was not much, possibly due to rather high operating temperature, i.e., 25.1 – 31.8℃. Yu et al. [7] reported that the effect of HRT on the acidogenesis was more significant than the effect of temperature. The CSTR treating whey wastewater had been operated at OLR 13.2 – 13.9 gCOD/L· d with influent COD 2,199 –

2,321 mg/L while the pH values in the system were varied at 4.18, 5.49 and 6.50. The typical variations of influent and effluent concentration are shown in **Figure 1**.

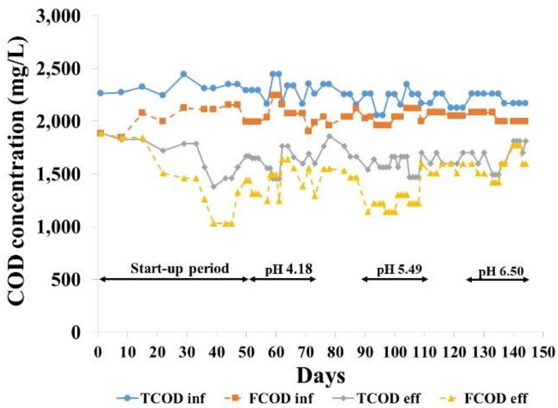


Figure 1. COD concentration variations of whey wastewater during the study

Alkali was added to the influent to raise pH values. However, the effluent pHs were always lower than the influent. The effluent pH 4.18 was initially planned for 4.50, but it suddenly dropped during the last period of investigation. The influent Filtrated Chemical Oxygen Demand (FCOD)/COD ratios of whey wastewater were in the range of 0.87 to 0.92 which were not much different from the effluent FCOD/COD ratios (0.78 – 0.96). The high FCOD/COD ratio indicated the high proportion of soluble organic matter. The effluent COD was not much different at each operating pH, i.e. 1,556 – 1,685 mg/L. The COD removals of whey wastewater in each operational pH condition were not much different, in the range of 23.6 to 33.0%. In the acidogenic reactor, major COD removal was not expected but the transformation of complex organics and VSS to solubilized VFAs and other intermediates [3].

Table 2: Performance comparison of whey, yeast and yolk wastewaters in the acidogenic CSTRs at various pH values

Parameter	Feed / pH	Whey				Yeast				Yolk			
		4.14 (1)	**4.18**	**5.49**	**6.50**	**4.50**	**5.57**	**6.30 (1)**	**7.09**	**4.55**	**5.52**	**6.25 (1)**	**6.56**
OLR	gCOD/L·d	13.8	13.9	13.3	13.2	13.0	12.9	14.2	14.2	13.5	13.1	13.2	14.7
Temp. (℃)	Influent	25.5±1.5	34.1±0.3	31.5±0.7	31.5±0.23	32.2±0.29	31.5±1.7	25.1±0.6	35.2±0.6	29.7±2.72	31.6±0.6	25.9±0.2	34.6±0.0
	Effluent	25.5±3.1	31.4±0.5	29.2±0.5	28.5±0.03	29.0±0.28	29.2±1.7	25.1±0.4	31.8±0.4	27.8±0.49	29.2±0.5	24.9±0.5	31.3±0.1
pH	Influent	6.62±0.1	4.46±0.05	6.83±0.08	6.91±0.07	4.56±0.12	5.35±0.1	6.45±0.04	7.52±0.08	5.08±0.05	5.77±0.05	6.64±0.14	7.59±90
	Effluent	4.14±0.1	4.18±0.12	5.49±0.07	6.50±0.03	4.50±0.08	5.57±0.1	6.30±0.06	7.09±0.08	4.55±0.03	5.52±0.08	6.25±0.28	6.56±99
COD (mg/L)	Influent	2,299±28	2,321±97	2,224±86	2,199±38	2,163±9	2,150±95	2,377±42	2,375±88	2,257±80	2,188±23	2,203±51	2,451±90
	Effluent	1,685±52	1,556±84	1,574±81	1,681±131	1,803±39	1,147±149	1,429±36	1,429±234	1,545±51	1,590±23	1,920±121	1,521±99
	% removal	26.7	33.0	29.2	23.6	16.6	46.7	39.9	39.8	31.5	27.3	12.8	37.9
FCOD (mg/L)	Influent	2,000±109	2,101±117	2,052±68	2,011±30	2,021±32	2,018±71	1,893±91	2,054±80	2,054±76	2,026±50	1,985±135	2,133±46
	Effluent	1,562±78	1,364±105	1,224±64	1,618±131	1,440±41	1,108±36	1,214±38	1,144±86	1,465±42	1,437±37	1,775±102	1,242±90
SS (mg/L)	Influent	363±20	411±34	238±43	336±21	73±11	60±92	326±7	203±11	788±52	1345±74	1903±52	1,262±96
	Effluent	201±35	401±115	235±21	206±28	42±6	54±19	150±8	ND	351±56	985±107	872±189	978±172
	% removal	44.6	2.4	1.3	38.7	42.5	10.1	54.0	ND	55.5	26.8	54.2	22.5
VSS (mg/L)	Influent	341±27	389±31	ND	305±21	64±9	58±74	288±7	193±3	742±47	1339±78	1718±73	1164±76
	Effluent	184±34	376±109	ND	200±37	38±6	51±18	136±7	ND	334±61	949±105	814±225	876±138
	% removal	46.0	3.3	ND	34.4	40.6	12.1	52.8	ND	55.0	29.1	52.6	24.7
VFA (mgHAc/L)	Influent	193±23	213±30	113±4	223±2	60±1	57±5	40±4	53±36	50±2	49±4	22±4	48±1
	Effluent	800±52	787±232	304±7	286±6	76±2	74±13	106±2	139±8	87±4	112±2	79±5	82±3
	% increase	314.5	269.5	169.0	28.3	26.7	29.8	165.0	162.3	74.0	128.6	128.6	70.8

Remark: NA = No data available, (1) = Results without pH adjustment [23]

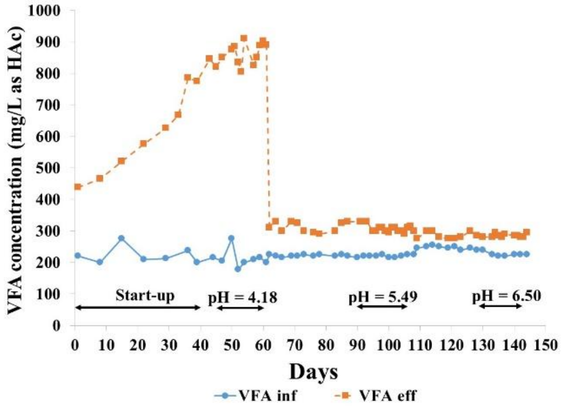
Yeast wastewater **(Table 2)**, with 2,150 – 2,377 mg/L of the influent COD and the FCOD/COD ratios of 0.80 – 0.94 were treated at the operational pHs of 4.50, 5.57, 6.30 and 7.09. The COD removal was rather similar (39.8 – 46.7%) at pH 5.57, 6.30 and 7.09 whereas, it dropped to 16.6% at the low pH (4.50).

The influent COD of yolk wastewater were in the range of 2,188 to 2,451 mg/L with FCOD/COD ratios of 0.87 – 0.93. The operating pHs of the system were adjusted at 4.55, 5.52 and 6.56, including 6.25 from the non-pH control condition. The COD removal varied without a distinct pattern at 12.8 – 37.9% with the maximum value of 37.9% at pH 6.56. The influent and effluent FCOD/COD ratios were higher than 0.90 except at the pH 6.56. Besides methanogenesis which was minimal in this study, it was still unknown on which reactions or mechanism be responsible for COD removal.

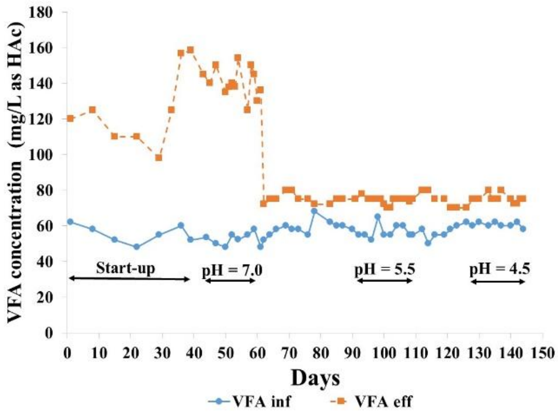
In whey wastewater, the influent SS concentrations were 238 – 411 mg/L with high VSS/SS ratios of 0.91 – 0.95 representing the high proportion of biodegradable solids. The SS and VSS removal of whey wastewater greatly fluctuated at 1.3 – 44.6% and 3.3 – 46.0%, respectively, with effluent VSS/SS ratios of 0.92 – 0.97. The influent SS concentrations of yeast wastewater were lower (60 – 326 mg/L) with the influent VSS/SS ratios of 0.88 – 0.97. The SS and VSS removal varied a lot at 10.0 – 54.0% and 12.1 – 52.8%, respectively with the effluent VSS/SS ratios of 0.90 – 0.94. Yolk wastewater had highest influent SS concentrations of 788 - 1,903 mg/L with the VSS/SS ratios of 0.90 – 0.99 which indicated the less soluble of yolk powder than whey powder and yeast extract. The SS and VSS removal of yolk wastewater fluctuated at 22.5 – 55.5% and 24.7 – 55.0%, respectively, with the effluent VSS/SS ratios of 0.90 – 0.96. The effluent VSS removal indicated that the organic matter in influent VSS was solubilized in the acidogenic reactor by the acidogenic microorganisms [27]. However, new cells of acidogenic bacteria also added up to effluent VSS concentration.

The biogas production of whey wastewater was found in small quantities at pH 4.18 and 5.49 as 6 and 3 mL/d, respectively, and none of the biogas production at pH 4.14 and 6.50. Yeast wastewater at pH 4.50 and 5.57 produced 1 and 3 mL/d of biogas whereas, at pH 6.30 and 7.09 gave no biogas. For yolk wastewater, the highest of biogas production was 5 mL/L at pH 6.25. The others pH had 1 – 3 mL/d of the biogas production. According to Hanjai et al. [23], methanogenesis was suppressed at HRT of 8 h or lower in the CSTRs treating whey, yeast and yolk wastewaters. In general, the acidification had a better performance with the less biogas production at the low HRT.

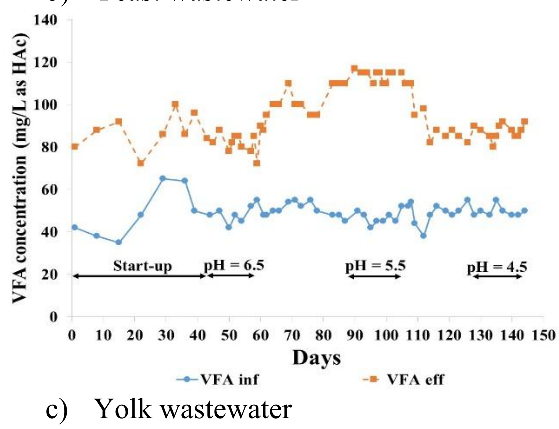
The variations of influent and effluent VFA concentrations of three substrates are shown in **Figure 2**. The effluent VFA was the result of the net value of VFA produced and consumed while the influent itself also contained VFA from protein substrate production. The average influent VFA concentrations of whey wastewater (**Figure 2(a)**) were the highest of the three substrates (113 – 223 mgHAc/L). The effluent VFA extremely increased to 800 and 787 mgHAc/L at pH 4.14 and 4.18, respectively whereas at pH 5.49 and 6.50 it slightly increased to 304 and 286 mgHAc/L, respectively.



a) Whey wastewater



b) Yeast wastewater



c) Yolk wastewater

Figure 2. VFA concentration profiles of all protein wastewaters during the study

The average influent VFA of yeast wastewater was 40 – 60 mgHAc/L while the average effluent increased to 74 - 139 mgHAc/L as presented in **Figure 2 (b)**. Yolk wastewater had the lowest VFA concentrations (22 – 50 mgHAc/L) whereas the average effluent VFA increased to 79 – 112 mgHAc/L (**Figure 2 (c)**).

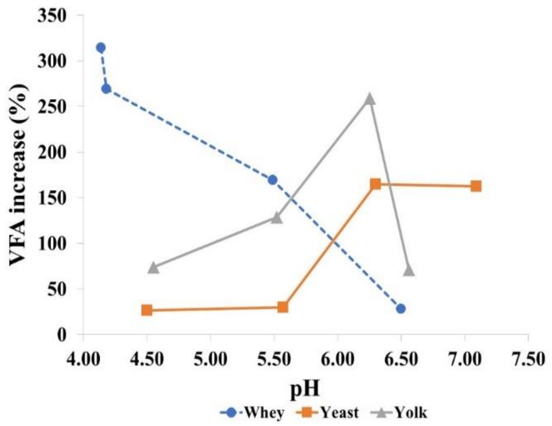


Figure 3. VFA increase of whey, yeast and yolk wastewaters in the acidogenic CSTRs at various pH values

The percentage of VFA increase during the study is shown in **Figure 3**. Low pH was found to be favourable for whey wastewater acidification with a distinct pattern of an increasing percentage of VFA production towards lower pH. The maximum percentage of VFA increase was 314.5% at pH 4.14, a condition without pH adjustment of the influent. The percentage decreased to 169.0% and 28.3% while the pH of the system was reached to 5.49 and 6.50, respectively. The acidic pH range enhanced the increasing of VFA production more than the neutral pH range in whey wastewater. Bengtsson et al. [17] found the optimum pH at 5.25 – 5.50 for VFA production in the acidogenic reactor of dairy and cheese-whey wastewater.

The percentage of VFA increase of yeast wastewater sharply rose as pH approaching neutral range, i.e. 165.0 and 162.3% at pH 6.30 and 7.09, respectively. Yu and Fang, [5] found the high VFA production at pH 6.00 – 6.50 and 12 h HRT in the acidification of the gelatinous wastewater. On the contrary, the study of pharmaceutical wastewater which had a high protein similar to yeast, using the acidogenic CSTR at OLR 14 gCOD/L·d of Oktem et al. [28], the highest acidification efficiency was found at the operational pH 5.50. At acidic pH range (4.50 and 5.57) of yeast wastewater, the percentage of VFA increase was rather stable at 26.7 – 29.8%.

The percentage of VFA increase of yolk wastewater rose up at higher pH to a maximum of 259.1% at pH 6.30. It was then dropped to 70.8% at pH 6.56. The acidic condition was suitable for the acidogenesis of whey and yolk wastewaters whereas the neutral condition was worthy for yeast wastewater. Elefsiniotis and Oldham [29] reported that the variation of pH between 4.30 and 5.20 did not affect VFA production and COD solubilization of primary sludge, but higher pH level (5.90 – 6.20) affected both parameters in acidogenesis. In additional, Demirel and Yenigun [16] found the higher pH levels from 6.00 to 8.00 affected the dominant microbial populations in an acid reactor treating dairy wastewater. According to the results obtained as presented in **Table 2**, the highest percentage of VFA increase in all wastewater occurred under influent without pH adjustment. It can be concluded that pH adjustment is not necessary for whey, yeast and yolk wastewaters.

The VFA compositions were calculated from individual acids, analyzed by GC over total VFA which was analyzed by titration. The essential VFA products produced in the acidogenic phase are critical because they affect methanogenesis efficiency [30]. The total VFA composition in this study were less than 100% which revealed that other intermediates were also present in the experiment. The main VFA compositions of the influent whey wastewater were 31 – 53% butyrate, 22 – 30% acetate and 11 – 17% propionate at all pH conditions except at pH 6.50 which had no acetate. For yeast wastewater, VFA consisted mainly of 29 – 63% propionate, 15 – 46% butyrate and 8 – 11% *i*-butyrate and *i*-valerate. In the meantime, the VFA composition of the influent yolk wastewater fluctuated with various VFAs such as *i*-valerate, *i*-butyrate, acetate and propionate. The effluent VFA compositions of whey, yeast and yolk wastewaters are shown in **Figure 4.** The complex substrate was broken down into the fermentation products modified to the consecutive methanogenic reactor. Short-chain acids such as acetate, propionate, butyrate and valerate were found to be the main product under fermentation.

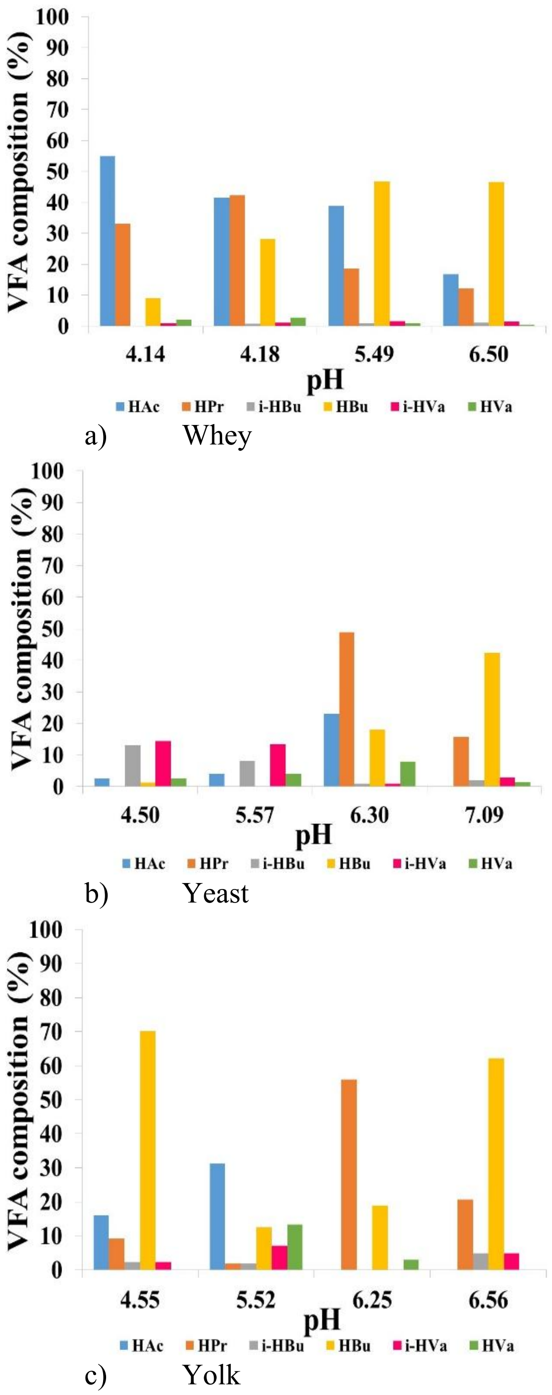


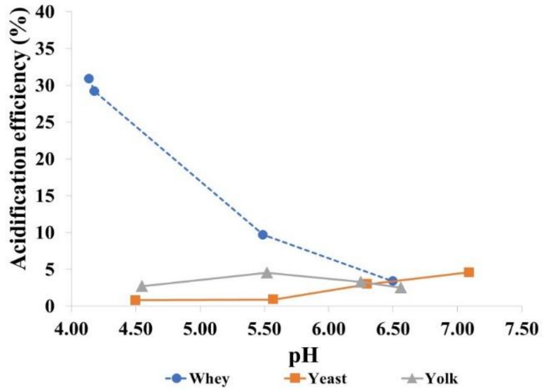
Figure 4. VFA composition in the effluent of whey, yeast and yolk wastewaters at various pH values

In the effluent of whey wastewater, acetate and propionate were found as the predominate with butyrate as the inferior at the acidic pH (4.14 and 4.18). At increasing pH (5.49 and 6.50) butyrate was the main VFA composition with acetate and propionate as the inferior. At all pH conditions of whey wastewater the small quantities of *i*-butyrate, *i*–valerate and valerate were found except at pH 4.14. In general, the acidic pH favoured the production of alcohols whereas the neutral pH favoured VFA in the acidification of lactose [31]. Zoetemeyer et al. [32] investigated glucose acidification and found that the VFA distribution was not influenced by the pH change. Acetate, butyrate, and *i*-butyrate were the main VFA products at pH 6.00 - 6.50 but at pH 4.50, they were propionate and ethanol. The distribution of product distribution was possibly due to the shift of the microbial population in the reactor [33].

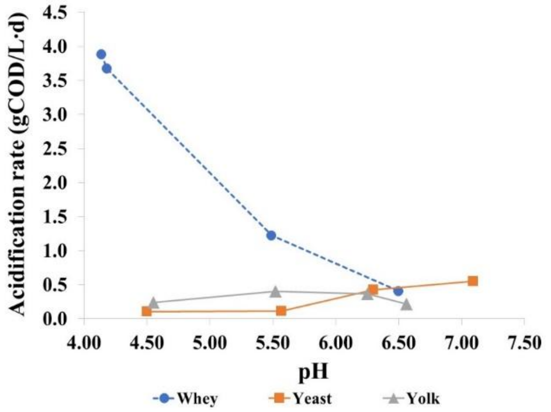
Yeast wastewater had an extreme shift of VFA composition in the effluent with *i*-valerate and *i*-butyrate as the main VFA products with the small quantities of acetate, butyrate, and valerate at acidic pH (4.50 and 5.57). At the neutral pH (7.09) butyrate and propionate were predominant with fewer quantities of *i*-butyrate, *i*-valerate and, valerate. At pH 6.30 propionate was the main VFA composition with acetate and butyrate as inferior.

Meanwhile, The predominant VFA compositions of the effluent yolk wastewater varied in each pH conditions which was butyrate at pH 4.55 and 6.56, acetate at pH 5.52 and propionate at pH 6.25. Butyrate and propionate were found in all pH conditions whereas acetate was found only at the acidic pH condition (4.55 and 5.52). The composition of wastewater was the fundamental characteristic which affected the VFA product and needs to be taken into account when VFAs were produced [34]. Carbohydrate is the main organic matter in the fermentation substrate which found in whey powder. The polysaccharides were firstly degraded to glucose, then pyruvate which was an important intermediate product before utilized to produce acetate and butyrate by the enzyme actions [35]. Protein has been found to be degraded faster than lipids during the hydrolytic-acidogenic stage but slower than carbohydrate [36].

The acidification efficiency and acidification rate obtained during the steady state of all protein wastewaters under various pH values are shown in **Figure 5**. Whey wastewater was the most sensitive to operational pH than yeast and yolk wastewaters with the performance sharply dropped at higher pH condition. The maximum acidification efficiency and acidification rate were found at pH 4.14 to be 30.9% and 3.88 gCOD/L· d, respectively. The values steadily decreased to 13.2% and 0.4 gCOD/L· d at pH 6.50.



a) Acidification efficiency



b) Acidification rate

Figure 5. Acidification efficiency and acidification rate of whey, yeast and yolk wastewaters at various pH values

It is hypothesized that a high proportion of carbohydrate (75.9%) in whey powder plays an important role in acidification. The higher acidification efficiency found in dairy effluent or synthetic dairy wastewater was realized as the results of carbohydrate and other easily degradable contents which found to be hydrolysed faster. However, yeast wastewater had an opposite trend with slightly increasing values at higher pH.

The acidification efficiencies and acidification rates in acidic conditions (pH 4.50 and 5.57) were not much different, but they increased to 4.6% and 0.52 gCOD/L· d at pH 7.09, respectively. The slightly acidic pH was suitable for the acidification of yolk wastewater. The maximum acidification efficiency (4.6%) and acidification rate (0.38 gCOD/L· d) were found at pH 5.52 while pH 6.25 also gave a slightly lower performance. The acidification efficiencies at pH 4.55 and 6.56 dropped to 2.7% and 2.5%, respectively. Comparison of three protein wastewaters indicated that the acidification efficiency was significantly higher for whey wastewater at the acidic pH. The neutral pH was favourable for yeast wastewater whereas the slightly acidic pH benefited to the yolk wastewater. According to, ANOVA at individual confidence level = 98.93% the performance of the study without pH adjustment, i.e. pH values for whey, yeast and yolk wastewaters at 4.14, 6.30 and 6.25, respectively, were better than the conditions with adjusted pH in the system. The pH adjustment is therefore not necessary for protein wastewaters as recommended by some research work [5, 28]. In the protein wastewater treatment, there is a naturally high buffer incapacity to resist pH change and pH adjustment is not beneficial in terms of the economic aspect. The acidogenic CSTR is an important part of the two-phase anaerobic treatment. This study confirmed the effect of pH on acidification of protein wastewater. The result obtained can help to optimize the design and operation of full - scale CSTR.

4. Conclusions

Based on the results obtained, the following conclusions can be drawn. At the same range of COD concentration, whey wastewater had high VFA while yeast wastewater had high SS concentrations. Whey wastewater was the most sensitive to operational pH with decreasing acidification efficiency and acidification rate at higher pH. Yolk wastewater preferred slightly acidic condition while yeast wastewater was better acidified at neutral pH range.

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