

Optimization of Two-Stage and Single-Stage Anaerobic Reactors Treating Cheese Whey

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ABSTRACT: The objective of this research is to compare and evaluate the methane production from cheese whey in single-stage and two-stage anaerobic treatment processes. Single stage-reactor was operated in a pilot-scale batch reactor while two-stage reactor was operated in batch-dilution and semi-continuously. Reactors were loaded with cheese whey with an chemical oxygen demand (COD) of about 38.61 g/L. Single stage system was operated at 35°C with a hydraulic retention time (HRT) of 15, 10, 7 and 4 days; respectively. The maximum methane production in the single-stage reactor was obtained at 3.86 gCOD/L.d organic loading rate (OLR) with 10 days HRT being about 0.39 L CH₄ /L_{reactor}/g COD_{deg.d}; respectively. The results indicated that the use of a two-stage process for anaerobic treatment of cheese whey resulted in 4 fold higher methane yield compared to single-stage reactor system allowing better control of the acidogenic and methanogenic phases.

Keywords: *Anaerobic treatment, Biogas, Cheese whey, Hydraulic retention time, Methane production, Phase separation*

Peyniraltı Suyu Arıtımında Tek ve Çift Aşamalı Anaerobik Reaktörün Optimizasyonu

ÖZET: Bu çalışmanın amacı tek ve çift aşamalı anaerobik arıtım proseslerinde peyniraltı suyunda metan üretimini araştırmak ve karşılaştırmaktır. Tek aşamalı reaktör pilot ölçekli kesikli reaktörde işletilirken, çift aşamalı reaktör yarı sürekli ve kesikli olarak işletilmiştir. Reaktör, 38,61g/L kimyasal oksijen ihtiyacına (KOİ) sahip peyniraltı suyu ile yüklenmiştir. Tek aşamalı sistem 15, 10, 7 ve 4 günlük HRT'ler ile 35°C'de işletilmiştir ve maksimum metan üretimi HRT'nin 10 gün olduğu işletme koşulunda 3,86 gKOİ/L.gün seviyelerine ulaşmıştır (0,39 L CH₄ /L_{reaktör}/g KOİ_{deg.gün}). Çalışma sonuçları peyniraltı suyunun anaerobik arıtımında asidojenik ve metanojenik fazların daha iyi kontrol altına alınabildiği iki aşamalı prosesin tek aşamalı prosese göre 4 kat daha fazla metan üretim verimi elde edilebildiğini göstermiştir.

Anahtar Kelimeler: *Anaerobik arıtım, Biyogaz, Peyniraltı suyu, Hidrolik bekleme süresi, Metan üretimi, Faz ayrımı*

1. INTRODUCTION

Cheese whey is a by-product of dairy industry, which is formed after settlement of the casein. This by-product generates about 85-95% of the milk volume and comprises 55% of milk nutrients. Cheese whey generally contains lactose, soluble proteins, fats and mineral salts and differs according to the procedure used for casein precipitation [1, 2]. If the pH value is lower than 5, it is called "acidic" cheese whey, as contrary; it's pH is among 6-7, it is called "sweet" cheese whey [3]. Acidic cheese whey has limited usage due to the high salt concentration and acidity [4, 5]. Through the production of 1 kg of cheese, 9 L cheese whey is formed [6]. Cheese whey production across the globe is 160 million tons [7]. Cheese whey has a

value of biological oxygen demand (BOD₅) between 30-50 g/L and chemical oxygen demand (COD) between 60-80 g/L. Due to the high organic matter concentrations and limited reuse, much attention has been focused on the effective treatment of acidic cheese whey. Biological methods are commonly considered to be the most effective treatment applications since they present lower operational costs and improved applicability. Anaerobic treatment is the only practical biological method for treating this wastewater, not only reduce the wastewater pollution but also can produce methane gas which is a valuable and renewable energy source. Previous studies have indicated that cheese whey can be effectively used for anaerobic treatment

[8]. The anaerobic treatment process generally involves two different sets of activities called acidogenesis and methanogenesis; respectively. In the first step (acidogenesis) complex organic matters are converted to simple ones by acid producing bacteria (APB), such as; conversion of lactose into volatile fatty acids (VFAs) which leads a rapid decrease in the pH of the media. This step relies on fermentation process; and acetate, butyrate, propionate, formic acid, carbon dioxide and hydrogen are among the most known end-products. The biodegradation step mainly occurs in the second step (methanogenesis) in which the end-products of the first step are converted into methane and carbon dioxide by methane producing bacteria (MPB) allowing wastewater to be used for the production of energy in the form of biogas. Conventionally, the first step and the second step of the anaerobic digestion are both performed in a single reactor in which different bacterial groups are to be live together. However, there is often an acidic environment in single-stage anaerobic reactors due to the acid production during acidogenesis phase, which may be inhibitory to MPB. Not only the low pH, but also short HRT may have inhibitory effect on MPB, contrarily; may be favorable conditions to the growth of APB, resulting in insufficient treatment and low methane production yield. Studies have showed the possibility of using two-stage anaerobic reactors by separating acid and methane producing microorganism, thereby; increasing the biogas production efficiency and process stability [9-13]. Two stage reactors allow independently controlling each stage and provide opportunity to optimize growth of APB and MPB.

Conventional anaerobic treatment of cheese whey seems quite problematic due to the tendency to acidify very rapidly. In recent years, several investigators have studied phase separation in anaerobic treatment of cheese whey and many stated that phase separation has increased overall stability, COD removal rate and methane formation [8, 14]. The phase separation by using HRT is a good option

for slow-growing MPB and fast-growing APB. The primary purpose of this study is to optimize the single and two-stage anaerobic reactors and investigate the phase separation strategy by using HRT. For this aim, single-stage system was operated in pilot scale at a HRT of 15, 10, 7 and 4 days; respectively. Afterwards, separate acidogenic and methanogenic reactors were optimized independently to be able to achieve high methane production yield.

2. MATERIAL AND METHODS

2.1. Process Descriptions and Experimental Set-Up

Single stage process was performed with a pilot scale batch reactor. The reactor was operated with a volume of 120 L at 35 °C. Agitation was provided with a motor (Inoxpa, Turino, Italy) and pH was adjusted to 7.2 with peristaltic pumps (Pentax, Veronella, Italy). The effluent was withdrawn in the middle of the reactor, leaving a working volume of 60L at the beginning of the next cycle.

The components of the two-stage process were 1L of acidogenic batch reactor and 5L of methanogenic batch reactor. The reactors were operated in batch-dilution and semi-continuously (Fig.1). The mixing in the acidogenic reactor was performed by magnetic stirrers and reactor was operated under room temperature (25°C) with and without pH control. The methanogenic batch reactor consisted of a 6.5L vessel (Bioflo 110, New Brunswick Scientific Co., Edison, NJ, USA) with an effective working volume of 5L. The contents of the reactor were mixed by a single shaft impeller system at a speed of 150 rpm. An internal pH controller (Bioflo 110 system) was used to control the pH between 7.2 and 7.3 by adding 0.2 M HCl and 0.2 M NaOH. The temperature was maintained at (35±0.5)°C.

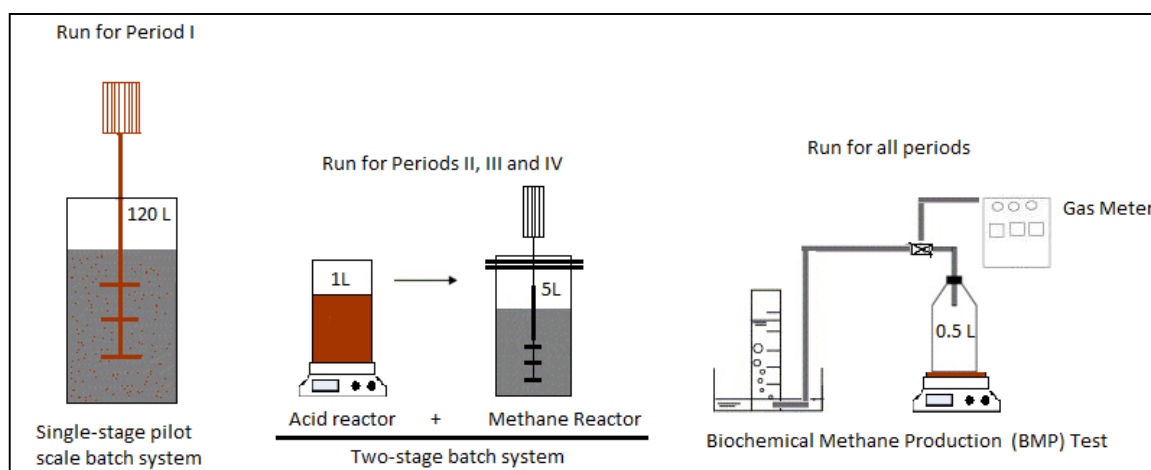


Figure 1. Schematic diagram of the single-stage, two-stage batch reactors and BMP test

BMP testing was done to evaluate gas production rate and methane yield during each study period. Biochemical methane potential (BMP) experiments were run in parallel with single- and two-stage batch systems to accurately measure the produced methane gas (Fig.1). BMP experiments always run in parallel with single and two-stage (for methane reactor) systems and inoculum was renewed each cycle from the continuous reactor. BMP experiments were

carried out in duplicates at 35 °C in 500 ml bottles. Gas production was determined with a glass syringe by the volume displacement technique. The amount of biogas produced and its methane content was measured periodically.

The performance of the single-stage and two-stage reactors was evaluated with over seven different periods (Table 1).

Table 1. Operational conditions

Periods		Temperature, °C	pH	HRT, day
I	Single-stage pilot reactor	35	7.2	15
II	Single-stage pilot reactor	35	7.2	10
III	Single-stage pilot reactor	35	7.2	7
IV	Single-stage pilot reactor	35	7.2	4
V	Two-stage acid reactor	25	No control	1
	Two-stage methane reactor	35	7.2	4
VI	Two-stage acid reactor	25	7.0	1
	Two-stage methane reactor	35	7.2	4
VII	Two-stage acid reactor	25	7.0	1
	Two-stage methane reactor	35	7.2	6

During Periods I-IV, the effect of HRT on anaerobic treatment of cheese whey in a single-stage reactor was investigated. In this period HRT was decreased from 15 days to 10, 7, 4 days; respectively. In Periods V-VII, two-stage anaerobic process was used to optimize independently acidogenic and methanogenic reactors to be able to achieve high methane production yield. In the Period V, acidogenic reactor was operated with a one day HRT at 25°C without pH control, however; methanogenic reactor was operated with four days HRT at 35 °C and pH was adjusted to 7.2. In Period VI, acidogenic reactor was operated with pH control; methane reactor had the same operational conditions as Period V. In the last Period, the HRT of the methane reactor was increased to 6 days. The operational conditions were changed after observing

steady state conditions. The reactors were considered to be running stable when for at least three days, total organic matter removal, organic acid production, and methane production profiles within a cycle were constant. All assays were run in triplicate and the data illustrated in all figures are the mean values of the measurements for 3 cycles.

2.2. Cheese Whey and Anaerobic Seed Culture

Digester sludge from a municipal wastewater treatment plant (Gaziantep Wastewater Treatment Plant, Gaziantep, Turkey) was used as the inoculum. The acid cheese whey was obtained from the AYDA cheese factory (Kahramanmaraş, Turkey). Initially, the pilot scale reactor was started with the addition of 60L of acid whey, followed by inoculation with 720 g of digester sludge. This

reactor was operated at 15 d HRT under batch conditions for 60 days to set up a stable anaerobic culture. After single-stage reactor studies were completed, anaerobic sludge in the pilot scale reactor was used as inoculum for the two-stage anaerobic reactors. Two-stage reactor was operated 15 days to set up a stable acidogenic and methanogenic culture.

Table 2. Composition of raw cheese whey

Parameter	Average \pm S.D
pH	4.56 \pm 0.1
Alkalinity, (g CaCO ₃ /L)	1.76 \pm 0.05
Total Organic Carbon, (g/L)	14.36 \pm 3.2
Total Chemical Oxygen Demand, (g/L)	38.61 \pm 4.2
Total Nitrogen, (g/L)	1.03 \pm 0.82
Suspended Solids, (g/L)	7.9 \pm 0.03
NH ₄ -N, (mg/L)	35 \pm 0.03
PO ₄ -P, (mg/L)	472 \pm 0.04

The chemical composition of the cheese whey was presented in Table 2. The distinctive characteristics are its high organic content and low pH value.

2.3. Analyses

The methane content of the biogas was determined using a portable Cosmos model XP-311 gas meter (Cosmos, Osaka, Japan). The total dissolved organic carbon concentration was determined by a TOC analyzer (Teledyne Tekmar, Torch, USA) equipped with an auto sampler. An ion chromatography (Dionex ICS-3000) was used to measure the concentrations of ions (Dionex, Sunnyvale, CA, USA) with Ion Pac AG19 guard and AS19 analytical columns. Eluent containing 8mM sodium carbonate Na₂CO₃ and 1.5mM sodium hydroxide (NaOH) was prepared and used for ion analyses with a flow rate of 1 ml/min. Volatile fatty acids (VFAs) were measured by HPLC 3000 liquid chromatography (Thermo Scientific, California, U.S.A.) with OA column at 0.6 ml-1 flow and, with methanesulfonic acid as liquid phase. Alkalinity, NH₄-N, pH, SS and volatile suspended solids (VSS) were determined by standard methods [15].

COD was determined according to APHA standard methods [15]. Samples were acidified with

concentrated H₂SO₄ to below pH 2 and purged with N₂ gas for approximately 5 min to remove H₂S prior to COD determination.

3. RESULTS AND DISCUSSION

3.1. Single-Stage Pilot-Scale Reactor Performance

3.1.1. COD Removal

The operational conditions applied to the single-stage pilot reactor (Period I, II, III and IV) are shown in Table 1. The temperature and pH of the system were kept at 35 °C and 7.2 and only HRT was varied during single-stage reactor studies. COD removal performance and alkalinity production profile of the single-stage reactor is shown in Fig.2.

The COD concentration of influent (raw cheese whey) was about 38.61 g/L during all operational conditions. In Period I, single-stage pilot scale reactor was operated at a HRT of 15d corresponding to organic loading rate of 2.57 gCOD/L.d. During this period, the COD removal in the reactor was at 34 \pm 5 %. However, COD removal at the end of the anaerobic period changed as the duration of the period was decreased, being approximately 33 \pm 6 % for the HRT of 10d (Period II), 53 \pm 5 % for the HRT of 7d (Period III), and 46 \pm 4% for the HRT of 4d (Period IV). Some fluctuation was observed during operational conditions. It was observed that after 4d of operation the COD value in the reactor started to increase probably due to the release of organic substances (expressed here as COD) as an effect of cell lysis led to a substantial increase in COD except for the 4-day duration. Another reason for this increase might be the solubilisation of particulate matter in the cheese whey. In a study performed by Göblös et al. [8] it was found that decrease in HRT from 40 days to 5 days generally led to a higher COD in the effluent, and COD reduction of between 95–68% was attained. During the study, the increase of pH in the reactor was observed as a result of alkalinity generation by the anaerobic biodegradation of organic compounds contained in the cheese whey as depicted in Fig.2.

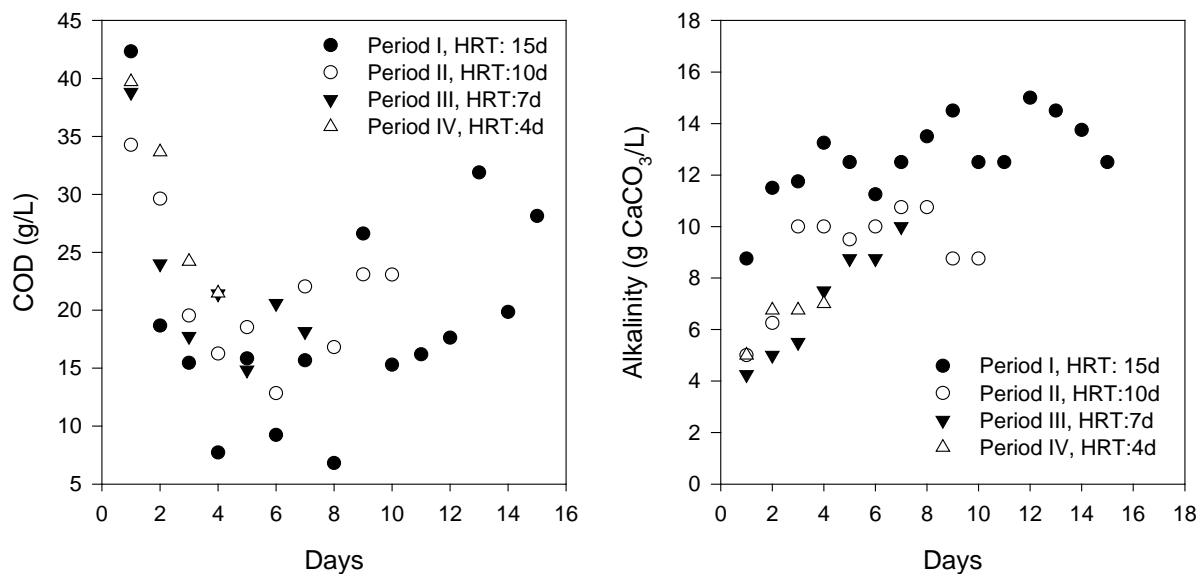


Figure 2. Effect of HRT on COD removal and alkalinity concentrations in single-stage system

The alkalinity production was highest in the Period I (HRT,15d) being about 15 g CaCO_3/L . In general, methane gas is produced from H_2/CO_2 or acetate in the methanogenesis phase in which acetate is a key intermediate metabolite [16]. There are two important pathways involved in methanogenesis; acetoclastic and non-acetoclastic methanogenesis; respectively. If methane is produced by acetoclastic methanogens acetate is converted to CH_4 and CO_2 and alkalinity is produced. On the other hand; in the non-acetoclastic pathway acetate is first oxidized to CO_2 and then the produced CO_2 is reduced to CH_4 by acetate-oxidizing bacteria and hydrogenotrophic methanogens (H_2 -oxidizing); respectively. Tendency of alkalinity raise in the anaerobic reactors give a presumption for the methanogenic pathways. According to our results the alkalinity production observed in the reactor can be explained by both the volatile fatty acids (VFA) converted to CH_4 and CO_2 by methanogens as well as the alkalinity generated by the anaerobic biodegradation of nitrogenous organic compound [17].

3.1.2. Composition of VFAs

VFAs such as acetate, propionate, butyrate, iso-butyrate, valerate and iso-valerate have long been recognized as the most important intermediates in the anaerobic process in which acetate has been

known as a key intermediate metabolite during methanogenesis [18]. The decomposition of VFAs is considered to be the rate-limiting step of the methane production [19]. Therefore, the composition of the VFAs in the biogas reactor under different operational conditions (Period I, II, III, and IV) was also determined and results are shown in Fig.3. The main products of this phase were acetic, propionic and butyric acids, however; valeric and isobutyric acids were not detected.

As seen in Fig.3. acetate was the dominant intermediate metabolite detected in our study except Period I (HRT,15d), in which propionic acid was detected at higher concentrations than acetate. Acetate concentration was the highest in the Period II (HRT, 10d) being about 2000mg/L. It was reported that the accumulation of propionic acid in the anaerobic processes result in low efficiency of the methanogenic phase due to the low acetogenic rate of propionic acid, hence the accumulation of propionic acid is not desired in the anaerobic process [20]. In these experiments the highest concentrations of acetic, butyric and propionic acids reached 1378, 823 and 1153 mg/L, respectively. The single-stage reactor in Period II (HRT, 10d) produced the highest total VFAs. Total organic acids were about 1.7, 2.7, 2.1 and 2.1 g/L in Periods I, II, III, and IV; respectively.

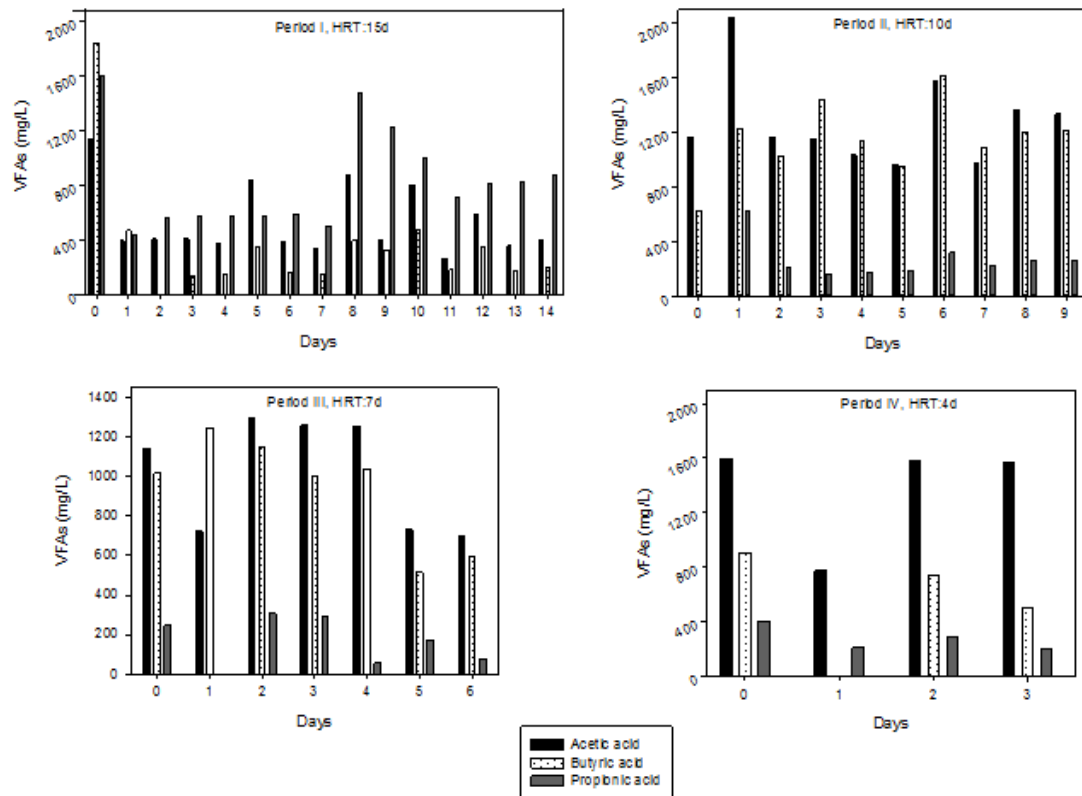


Figure 3. Effect of HRT on VFAs composition in single-stage system

3.2. Two-Stage Reactor Performance

The aim of this part of the study was to perform an anaerobic system with better running stability and higher working efficiency. Additionally, it was aimed to obtain maximum acidification to enhance methane production in subsequent methanogenic reactor. The operational conditions applied to the two-stage lab-scale reactor (Period V, VI, and VII) are shown in Table 1. The acidogenic reactor was operated at a HRT of one day for all operational conditions without pH control during Period I, with pH control (at 7) during Periods VI and VII. The methanogenic reactor was operated at a HRT of 4 d and 6 d for Periods V-VI, and VII; respectively.

3.2.1. COD Removal

For all operational conditions, the influent COD was almost the same being about 38.61 g COD/L. Fig.4. represents the COD removals in the two-stage anaerobic reactor under each of the operational conditions.

Acid reactor was inoculated with sludge taken from single-stage anaerobic reactor and operated without pH control to eliminate the

methanogens in the acid reactor since optimum pH conditions for methanogens (in the range of 6.5-8.2) are at higher values than acidogens. Additionally, the temperature was kept at 25 °C for all periods since it was known that the number of methanogens decreased significantly with the drop in temperature.

At the start-up period, the HRT was 0.5 day which resulted in washing out of the system as a result of rapid decrease in pH values resulted from the tendency of cheese whey to acidify rapidly. The observed pH values in acid reactor were in the range of 2-3.5. It can be concluded that although acidogens are less sensitive to low pH conditions than methanogens the low pH values (in the range of 2-3.5) were not the optimum for acidogenic bacteria. Then, HRT was increased from 0.5 day to one-day. Actually lower HRT values favored the production of VFAs, however; higher values stimulated methanogenic activities, mainly 12h was found optimum for VFAs production [21-23]. The system was recovered as a result of increasing HRT to one-day and most of the methanogens were successfully inhibited (no methane was detected during acidogenic stage). The observed pH values were in the range of 3.5-4.5 in this period. The pH of acid reactor was increased to 7 for Periods VI and VII after elimination of methanogens under low pH

values as acid reactor was more stable at higher pH values than 3.4-4.5. Actually, pH is one of the most important factors effecting the organic acid formation in acid reactor [24]. In a study performed by Yu and Fang [24] pH was found have a more significant effect on acidogenesis than that of temperature and they observed that optimum pH for the acidogenic activity is at 6-7. The results corresponding to VFAs production in acid reactor with varying pH values was introduced in subsequent chapter. Percentage COD removals in the acid reactor and methane reactor are shown in

Fig. 4. COD removal during the first step of bioconversion (acid reactor) was 2.5–8.5 g/L, corresponding to a COD removal of about 5-20%. COD removal efficiency in the methane reactor was 74%, 58%, and 56% for Periods V, VI, and VII; respectively. As observed in the single-stage studies, a slight increase in COD value was detected for the 6-day duration (Period VII) in methane reactor. The alkalinity was more stable in methane reactor compared to the single-stage pilot reactor and obtained about 9-10 g CaCO₃/L.

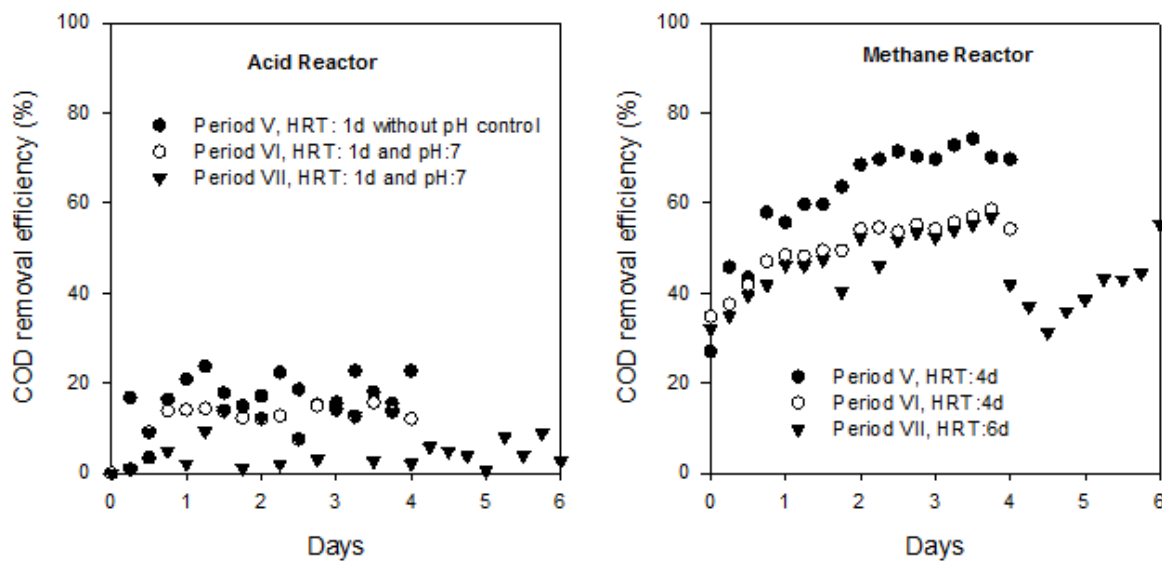


Figure 4. Effect of HRT and pH control on COD removal efficiency in acid and methane reactor in two-stage system

3.2.2. Composition of VFAs

The important products produced in the acidogenesis phase are very important since they can affect the efficiency of the methanogenesis phase [25]. In our study we observed the produced products during acidogenesis phase and the composition of VFAs in acid reactor was represented in Fig. 5. for each operational conditions.

Higher VFAs productions and variety of VFA types were observed with better running stability in acid reactor of two-stage system compared to the single-stage anaerobic reactor. In these experiments, acetic, butyric and propionic acids were the main VFAs in all of the periods and acetic, butyric and propionic acids reached to the highest concentrations in Period V (without pH control, HRT of 1d) being about 1568, 1747, and 2724 mg/L; respectively. The acidogens running without pH control produced the highest VFAs. Total organic acids were about 4.3, 3.7 and 2.5 g/L

for Period V, VI, and VII; respectively. It is important to note that the dominant VFA was not acetate in acid reactor of the two-stage system, however; propionic acid concentration was always high compared to the other VFAs in all periods. In the single-stage reactor the concentration of produced total VFAs was 2.7g/L which was lower than those in acid reactor of two-stage system. It has been reported that VFA production depends mainly on wastewater characteristics and operational conditions such as temperature, pH, and HRT [25, 26]. According to our results, the acid reactor can be characterized by its low pH, high VFAs and low COD removal as reported before [12]. Show et al. [27] proposed that the butyric acid/acetic acid ratio is a good indicator of hydrogen yield (not determined in this study), with reported values between 2.1 and 5.9. In our study, acid reactor show higher butyric acid/acetic acid ratios compared to single stage reactor as shown in Fig.5. This proposed the production of H₂ during acidogenic stage.

Determination of the VFA species in the anaerobic treatment is important, since it provides important information regarding the metabolic pathway of the process. The distribution of the metabolic products was different in single and two-stage experiments. Partial pressure of hydrogen has a vital role in methane formation and substrate flow. In other words, thermodynamically, the conversion of VFA to methane depends on the partial pressure of the hydrogen (that was hard to measure during our study). Most of the H_2 produced comes from oxidation of volatile and long chain fatty acids to acetic acid. Because of the thermodynamics of this reaction, it is inhibited by high partial pressures of H_2 . H_2 production by acetogens is generally energetically unfavorable due to high free energy requirements. An extremely low partial pressure of H_2 (10^{-5} atm) known to be a significant factor in propionate degradation to CH_4 . The partial H_2 pressure is normally regulated by H_2 -consuming (methanogens) and H_2 producing bacteria (acetogens). Since methanogens in the acid reactor during the two-stage system operation was washed out by pH regulation, H_2 pressure was probably increased since it was not consumed. Therefore,

butyric and propionic acid was dominant product during acid reactor. Anaerobic oxidation of propionate by acetogenic bacteria is only possible, if the hydrogen and formate concentrations are kept extremely low [28]. The majority of anaerobic propionate-oxidizing bacteria use the methylmalonyl-CoA pathway, yielding acetate, CO_2 and H_2 , but a second pathway is also possible which leads to butyrate and acetate production [28].

3.3. Comparison of One-Stage and Two-Stage Reactors in Terms of Methane Production Efficiency

Fig. 6 illustrates the methane yield and methane productivity profile obtained during one and two-stage configurations operated under different operational conditions. The calculated methane yield was expressed as the liter of methane produced per g of COD degraded per reactor volume per day, while methane productivity was expressed as the liter of methane produced per reactor volume per day.

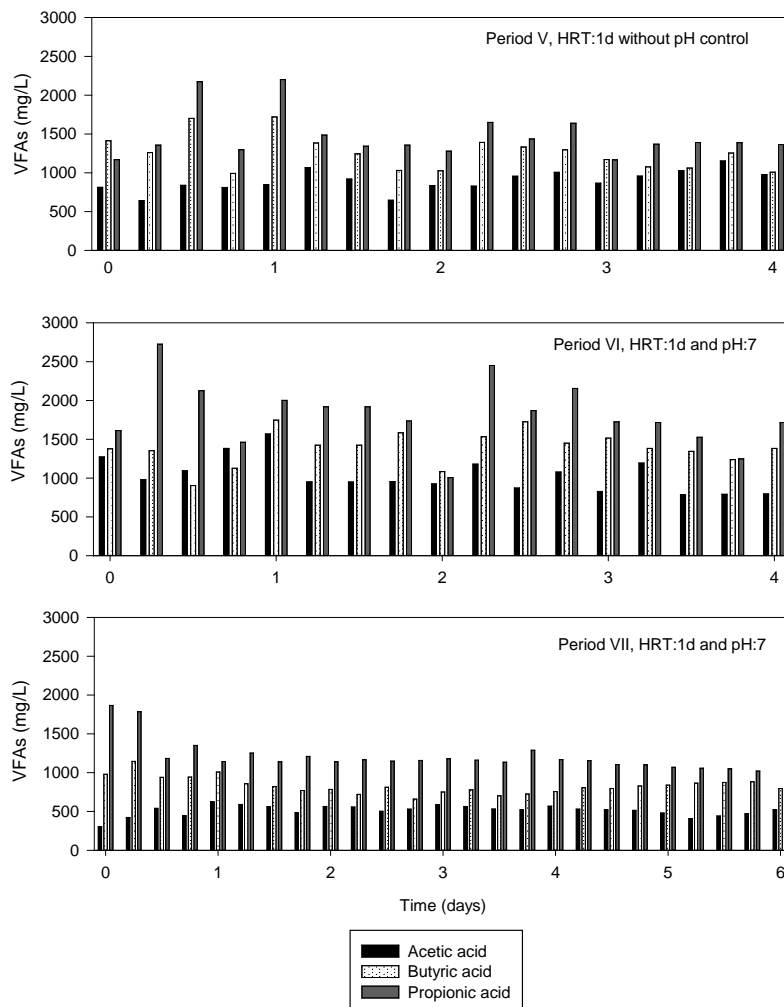


Figure 5. Effect of pH on VFAs composition in acid reactor in two-stage system

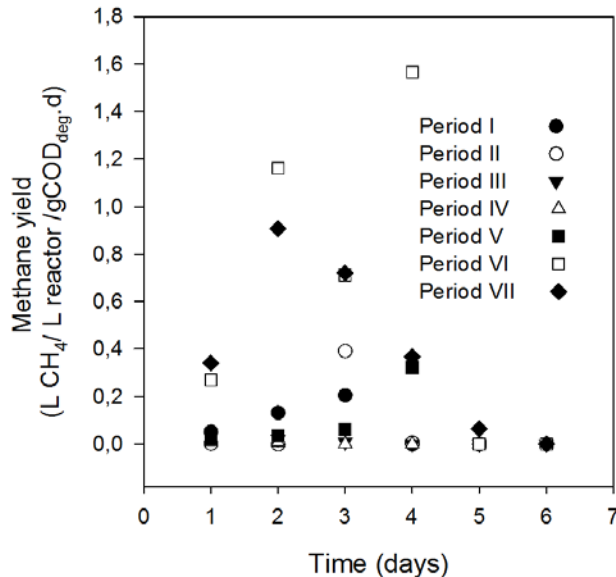
The maximum methane yield in the single-stage reactor system was $0.39 \text{ L CH}_4/\text{L}_{\text{reactor}} / \text{g COD}_{\text{deg.d}}$ (Period II; HRT:10d) while it was about $1.56 \text{ L CH}_4 / \text{L}_{\text{reactor}} / \text{g COD}_{\text{deg.d}}$ (Period VI) in two-stage reactor system.

Taking into account that theoretically 0.35 L of methane is produced per gram of COD removed, when assuming that all of the incoming COD is transformed into methane the effectiveness of the anaerobic reactor in converting cheese whey into methane (46% and 28% of theoretical value for two-stage and single-stage; respectively) at mesophilic temperature is also clearly demonstrated. The maximum methane productivity in the single stage reactor system was $0.5 \text{ L CH}_4/\text{L}_{\text{reactor}}/\text{day}$ (Period I; HRT:15d) while it was about $0.8 \text{ L CH}_4/\text{L}_{\text{reactor}}/\text{day}$ (Period VI) in two-stage reactor systems. As seen in Fig. 6, methane production in single-stage system increased with the decrease in HRT from 15-day to 10-day, but then start to decrease with decreasing HRT to 7-day and 4-day

which can be explained by insufficient duration for biodegradation of readily biodegradable substances. In two-stage system, phase separation was performed by using HRT and pH.

Acid reactor running with pH control showed better performance in terms of methane production and increasing HRT from 4-day to 6-day, the methane production was decreased which may be resulted from lower VFAs produced in the previous acid reactor. According to our results; the two-stage process clearly showed a better performance in terms of methane yield which was increased about 4 fold compared to single-stage reactor system allowing better control of the acidogenic and methanogenic phases. However, if the influent organic content is increased, the acidogenic activity, which includes the production of mainly VFA, CO_2 and H_2 , also increases which can result in an accumulation of organic acids and sudden decrease in pH in the reactor.

If this practiced in a single-stage reactor, the risk could be bigger since growth of the methane producing methanogens is inhibited below a pH of 6.6. The time required for organic carbon biodegradation to VFA and further methane is



increased, thereby resulting in increasing anaerobic reaction time. Determining the correct HRT is therefore critical to the optimization of reactor performance and maximizing methane production.

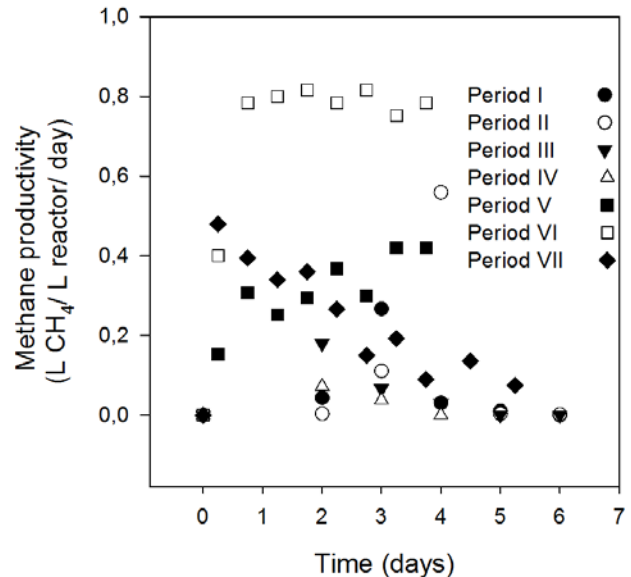


Figure 6. Effect of operational conditions on methane yield and methane productivity in single- and two-stage systems

Ratusznei et al. [29] used a stirred sequencing batch reactor (ASBR) for anaerobic whey treatment. They obtained high organic matter removal of about 96% at effluent concentration below 160 mg COD/L for non-filtered samples in this single-stage system. In a study performed by Damasceno et al. [30] the effects of feeding time and organic loading in an anaerobic sequencing batch biofilm reactor on whey treatment was investigated. They noted that when filling time was increased from 10 min to 2h and 4h, volatile acid concentration was increased. Furthermore, no significant differences were detected in the maximum concentration of total volatile fatty acids for any of the conditions investigated. However, the maximum values of propionic acid tended to decrease with increasing fill time considering the same organic load. Additionally, they observed that there was no tendency toward acid accumulation, indicating that 8h total cycle time remained insufficient for complete volatile acids consumption. Saddoud et al. [12] studied a system consisting of a stirred acidogenic reactor followed by a methanogenic reactor coupled with a membrane filtration system for the treatment of cheese whey. They achieved biogas methane content higher than 70% and biogas production 10 times higher than reactor volume. The removals of COD and BOD₅ were higher than 98%. In another study performed by Yang et al. [31] one and two-phase thermophilic

anaerobic digestion systems were used for cheese whey treatment. According to that of study results; rate of COD removal, rate of and yield of methane formation in the two-phase process were, respectively, 116%, 43%, and 6% higher than those of the single-phase system were.

4. CONCLUSION

In this study single-stage and two-stage anaerobic treatment of cheese whey was performed and effect of HRT was investigated in single-stage reactor. HRT of 10d was found optimum in terms of percentage COD removal (33%), biogas production yield (0.39 L CH₄ /L_{reactor}/g COD_{deg.d}) and biogas productivity (0.5 L CH₄/L_{reactor} /day). Total VFA concentration of 2.7 g/L was obtained. In two-stage reactor system, a maximum TVFA concentration of 4.3 g/L was achieved in the acid reactor at an HRT of 1 d without pH control. Two-stage reactor system led to an increase of TVFA production about 1.5 fold compared to single stage reactor system. The low COD removals were attained in acid reactor between 5-20% for all operational conditions. COD removal efficiency was 53% in single-phase system, which was increased to %74 in methane reactor of two-stage system. 4-day duration of methane reactor was found optimum for this study for effective COD removal and methane production. The methane

production efficiency is mostly dependent on acetate availability that can be optimized in acid reactor. The higher acetate production in acid reactor resulted in higher methane production in methane reactor. The two-stage process clearly showed a better performance in terms of methane yield, which was increased about 4 fold, compared to single stage reactor system.

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