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Anaerobic Co-digestion of Municipal Primary Sludge and Whey

A Dissertation Submitted in Partial Fulfilment of the
Requirements for the Masters Degree
in Environmental Engineering
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ABSTRACT

The aim of this research was to investigate the feasibility of co-digestion of municipal primary sludge and whey by anaerobic CSTR (Continuous Stirred Tank Reactor), as well as the factors that affect the performance of the co-digestion reactors.

Before studying the co-digestion process, a semi-continuous whey digestion experiment was conducted to analyze the feasibility of anaerobic digestion of whey along with pH control. The results obtained from the study indicated that supplement of nutrients, trace elements as well as heavy metals was necessary to maintain the anaerobic whey digestion system.

To investigate the co-digestion of primary sludge and whey process, the effects of pH, OLR (Organic Loading Rate), HRT (Hydraulic retention time) as well as the COD (Chemical Oxygen Demand) loading ratio of primary sludge to whey on the performance of the reactors were studied. The results of the co-digestion experiments demonstrated that it was feasible to co-digest primary sludge and whey without nutrient, trace element and heavy metal supplement. The TCOD (Total Chemical Oxygen Demand) removal efficiency and the biogas production of the co-digestion system increased with the increase of OLR. At same OLR, digestion of the mixture of primary sludge and whey with higher whey content achieved higher biogas production and TCOD removal efficiency. The anaerobic co-digestion of primary sludge and whey process performed successfully at OLR of 5.8 ± 0.1 g COD/l.d without pH control when the COD loading ratio of primary sludge to whey was approximately 70:30, due to the fact that the primary sludge may serve as buffering reagent. By adding sodium bicarbonate (NaHCO_3) to maintain the pH at 6.9 ± 0.1 , the OLR of the co-digestion reactor could reach 8.1 ± 0.1 g COD/l.d at HRT of 20 days. Moreover, by co-digestion of primary sludge and whey solution, the reactor could be operated successfully at HRT of 10 days and at OLR of 7.6 ± 0.1 g COD/l.d with COD loading ratio of primary sludge to whey of 53 : 47. The biogas production (3.2 ± 0.1 l/d) was 1.5 l/d higher than digestion of the same amount of primary sludge alone (1.7 ± 0.1 l/d).

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CHAPTER 1 GENERAL INTRODUCTION

1.1. Background of this study

Whey, a by-product of cheese or casein making, is the liquid remaining after the recovery of the curds, which are formed by the action of enzymes or acid on milk. It comprises 80-90% of the total volume of milk entering the process and contains more than half the solids present in the original whole milk, including 20% of the protein, most of the lactose, vitamins and minerals (Bylund, 1995).

Due to many small and medium-size dairy industries not having the economic incentive to further use whey, it is necessary to consider whey as a waste stream (Mockaitis et al., 2005). Whey makes up a significant part of environmental problem and contributes substantially to the pollution of surface waters and soil (Kosseva et al., 2003). Whey has a high organic content with a chemical oxygen demand (COD) of about 60-80 g/l, which leads to disposal problems (Patel & Madamwar, 1998). Because of the high organic content of whey, anaerobic digestion is essentially the only viable treatment method (Gavala et al., 1999). However, whey is a quite problematic substrate to treat anaerobically because whey has low bicarbonate alkalinity and tends to acidify rapidly due to its very high biodegradability (Malaspina et al., 1995). Supplemental alkalinity is required in order to avoid anaerobic process failure (Mockaitis et al., 2005). In addition, whey lacks nitrogen and phosphorous compared to the high COD content, and thus supplement of nutrients is necessary to maintain the digestion system (Gillies, 1974).

Municipal primary sludge contains a high level of nutrients and alkalinity. Provided that digester capacity was available, it would seem feasible to consider the co-digestion of whey in the existing municipal primary sludge digesters in WWTP (Waste Water Treatment Plant). By co-digestion of municipal primary sludge and whey, the sludge could provide nitrogen, phosphorous, trace metals and alkalinity, while whey could provide highly biodegradable organic content. The efficiency and the biogas volumetric

productivity of the digesters in WWTP may be improved, and at the same time, the disposal problem of whey in dairy industries may be solved.

Little study on anaerobic co-digestion of municipal primary sludge and whey has been done. Therefore, it is necessary to study the feasibility and the performance of the anaerobic co-digestion process.

1.2. Objectives of this study

The aim of this research was to investigate the feasibility of co-digestion of municipal primary sludge and whey by anaerobic CSTR, as well as the factors that affect the performance of the co-digestion reactors.

The research had the following objectives:

- (1) To investigate the feasibility of anaerobic semi-continuous digestion of whey alone.
- (2) To investigate the feasibility of anaerobic co-digestion of primary sludge and whey.
- (3) To investigate the effect of COD loading ratio of primary sludge to whey on the performance of the co-digestion system at same OLR.
- (4) To investigate the effect of pH on the stability of the co-digestion of primary sludge and whey process.
- (5) To investigate the achievable OLR of the co-digestion of primary sludge and whey process.
- (6) To investigate the effect of OLR and HRT on the biogas production and the TCOD removal efficiency of the co-digestion system.
- (7) To investigate the advantages of co-digestion of primary sludge and whey.

LITREATURE REVIEW

2.1. Anaerobic treatment

2.1.1. Anaerobic treatment process

Anaerobic treatment is a natural process in which a variety of different species from two entirely different biological kingdoms, the bacteria and the archaea, work together to convert organic wastes through a variety of intermediates into methane gas, an excellent fuel (McCarty, 2001). Anaerobic digestion involves the decomposition of organic matter and inorganic matter (principally sulphate) by anaerobic microorganisms in the absence of molecular oxygen. The three types of chemical and biochemical reactions that occur in anaerobic digestion are hydrolysis; fermentation, also referred to as acidogenesis (the formation of soluble organic compounds and short-chain organic acids); and methanogenesis (the bacterial conversion of organic acids into methane and carbon dioxide), as shown in Figure 2.1.

Hydrolysis is the first step of anaerobic digestion process. In the hydrolysis process, particulate material is converted to soluble compounds catalysed by enzymes that have been released to the medium by aerobic and anaerobic bacteria. The soluble compounds can then be hydrolysed further by anaerobic bacteria to simple monomers such as sugar, amino acids and fatty acids that are used by bacteria that perform fermentation.

Fermentation (also known as acidogenesis) is the second step of anaerobic digestion process. In the fermentation process, sugar, amino acids, and some fatty acids are degraded further to hydrogen, carbon dioxide, acetate, propionate and butyrate by acid-producing “fermentative” bacteria. The propionate and butyrate are also further degraded to the final products of fermentation (hydrogen, carbon dioxide, acetate) that are the precursors of methane formation (methanogenesis).

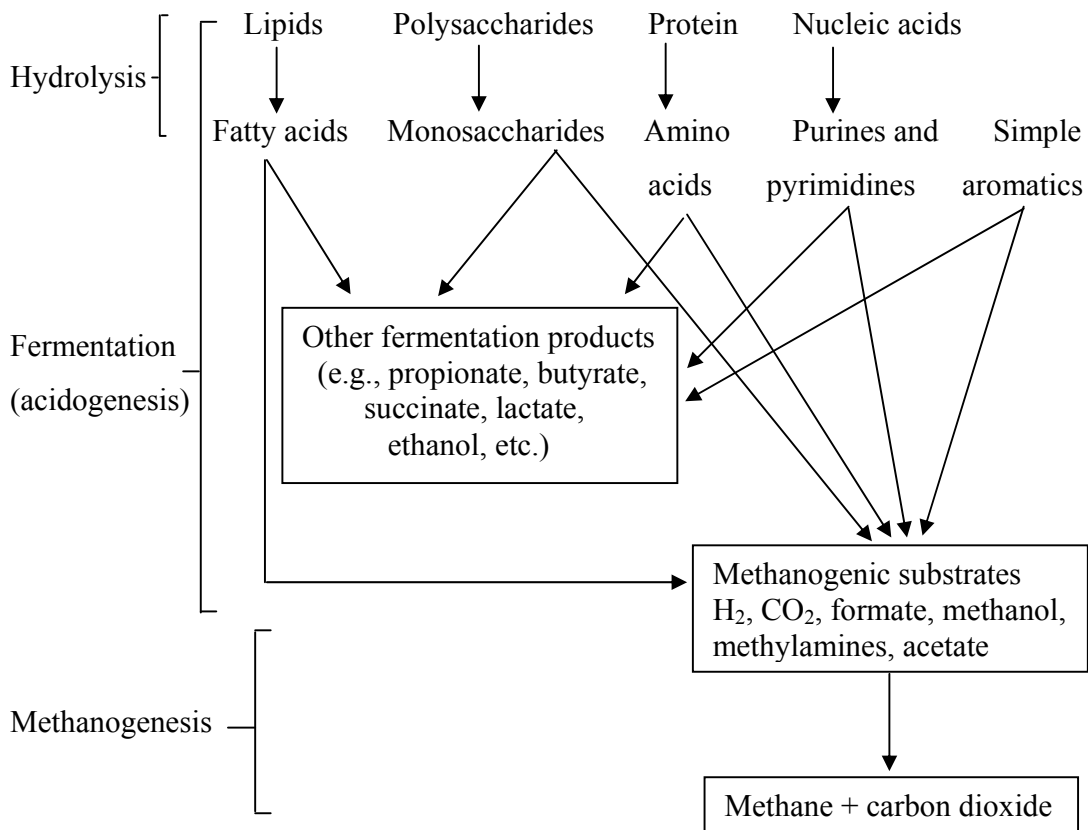


Figure 2.1 Anaerobic process schematic of hydrolysis, fermentation, and methanogenesis (Metcalf & Eddy, 2003).

Methanogenesis is the third step of anaerobic digestion process. In the methanogenesis process, the final products of fermentation (hydrogen, carbon dioxide, acetate) are degraded further to the final products of methanogenesis (methane and carbon dioxide) by methanogens.

Two groups of methanogenic organisms are involved in methane production. One group, termed *acetoclastic methanogens* (*Methanotrix* and *Methanosarcina*), split acetate into methane and carbon dioxide. The second group termed hydrogen-utilizing methanogens (*Methanobacterium*, *Methanobacillus*, and *Methanococcus*) use hydrogen and carbon dioxide to produce methane. The methanogens and the acidogens form a syntrophic (mutually beneficial) relationship in which the methanogens convert fermentation end products such as hydrogen, formate, and acetate to methane and carbon dioxide. The

microorganisms responsible for methane production, classified as archaea, are strict obligate anaerobes. The nonmethanogenic microorganisms responsible for hydrolysis and fermentation are facultative and obligate anaerobic bacteria (Metcalf & Eddy, 2003). The methanogenesis is, in most of the cases, the rate-limiting step of the overall process; however, hydrolysis could also be the rate-limiting step during the anaerobic digestion of wastewater rich in organic solids (Valentini et al., 1997).

2.1.2. Advantages of anaerobic treatment

There are significant economic and environmental advantages to utilize anaerobic waste treatment process. Some of the advantages include increased process stability, reduction of waste biomass, reduction of installation space requirements, conservation of energy with ecological and economical benefits, and minimization of operational attention requirements.

Anaerobic treatment has lower microbial yields by a factor of 6 to 8 times than aerobic processes, yielding reduced quantities of sludge that must be stabilized and disposed of in landfills or by composting. Sludge processing and disposal costs are reduced greatly (Speece, 1996).

The biogas composition in most municipal anaerobic digesters is about 70% methane, with the remainder being primarily carbon dioxide. Hydrogen sulphide, hydrogen, and carbon monoxide are also present in small amounts. Digester biogas is quite useful in reducing the in-house energy costs associated with operating a wastewater treatment facility. In cases where excess biogas is produced, it can be utilized off-site for additional heating and power after the removal of hydrogen sulphide (when concentrations exceed 7%) (Bailey and Ollis, 1985).

2.1.3. Disadvantages of anaerobic treatment

Despite many successes, potential disadvantages also exist for anaerobic treatment. The most significant negative factor that can affect the economics of anaerobic treatment is the possible need of alkalinity addition. To maintain an acceptable pH with the high gas phase CO₂ concentration, alkalinity concentration of 2000 to 3000 mg/l as CaCO₃ may be needed in anaerobic processes (Metcalf & Eddy, 2003). A significant cost may be incurred to purchase alkalinity if this amount of alkalinity is not available in the influent wastewater or cannot be produced by the degradation of proteins and amino acid.

2.2. Application of anaerobic treatment process

Suspended growth reactors, hybrid reactors and supported growth reactors are the main reactors that are currently in use for full-scale treatment applications. Suspended growth reactors include continuous stirred tank reactors (CSTR) and anaerobic contact reactors. Hybrid reactors include upflow anaerobic sludge blanket (UASB) reactors and upflow sludge blanket / fixed bed reactors. Supported growth reactors include fixed bed reactors and expanded / fluidized bed reactors (Eric, 1992).

In general the suspended growth reactors are advantageous for the treatment of sludges or wastewaters containing high proportions of particulate biodegradable material. The hybrid reactors are suitable for wastewaters with intermediate levels of particulates, although performance is usually better with soluble wastewaters. The supported growth reactors can be applied to wastewaters that contain primarily soluble organic substrates (Sutton, 1990).

The composition of the wastewater in terms of its particulate and soluble fractions affects the type of anaerobic reactor selected and its design. Wastewaters with high solids concentrations are treated more appropriately in suspended growth reactors than by upflow or downflow attached growth processes (Eric, 1992). Table 2.1. shows the comparison of these three anaerobic reactors.

Table 2.1 Comparison of suspended, hybrid and supported growth anaerobic treatment systems (Eric, 1992).

Factor	Suspended growth	Hybrid	Supported growth
Biomass concentration attainable	Low	High	High
SRTs attainable	Low	High	High
Suitable for wastewaters with particulates	Yes	Partial removal of particulates	Poor removal of particulates
Suitable for very concentrated wastewaters	Yes	Not suitable	Not suitable
Suitable for dilute wastewaters	No	Yes	Yes
Removal efficiency	Limited	High	High
Resistance to toxics and dynamic operating conditions	Limited due to short SRTs	Longer SRTs impart improved stability	Longer SRTs impart improved stability
Maintenance of internal hydraulic integrity	Relatively simple with mechanical mixing	Generally satisfactory with effluent recycle and evolved biogas mixing	Excess biomass accumulation can negatively impact reactor hydraulics
Power requirements	Generally lowest	Higher if effluent recycle practiced	Can be high if support medium is fluidized

The major applications of anaerobic digestion are in the stabilization of concentrated sludges produced from the treatment of municipal and industrial wastewater. Because of the emphasis on energy conservation and recovery and the desirability of obtaining beneficial use of wastewater biosolids, anaerobic digestion continues to be the dominant process for stabilizing sludge. The objective of anaerobic sludge digestion is the transformation of wastewater sludges to innocuous and easily dewatered substance. In the

process, a portion of the organic solids are microbiologically converted to methane and carbon dioxide gases (Joseph and Malina, 1992).

The single-stage continuous stirred tank reactor (CSTR) digestion process, which is a frequently used in modern sludge digester design, is characterized by heating, auxiliary mixing, uniform feeding, and thickening of the feed stream. The sludge is mixed by gas recirculation, pumping, or draft-tube mixers (separation of scum and supernatant does not take place), and sludge is heated to achieve optimum digestion rates. Because the total solids are reduced by 45 to 50 percent and given off as gas, the digester sludge is about half as concentrated as the untreated sludge feed (Eric, 1992).

2.3. Factors affecting performance of anaerobic digestion process

The anaerobic digestion system as a complex biological system is affected by several environmental factors. The most important environmental factors that can affect the balance of the system are discussed in the following sections:

2.3.1. Hydraulic retention time (HRT) and solids retention time (SRT)

The hydraulic retention time (HRT) is the average time the liquid is held in the digestion process. Standard hydraulic retention time in a municipal sludge CSTR system without sludge recycle is 15-20 days. However, a well-mixed and heated digester may produce good quality digested sludge after a 10-day retention time (Water Pollution Control Federation, 1987).

The biological solids retention time (SRT) is the average time the solids are held in the digestion process. For a CSTR system without recycle, $SRT = HRT$. The performance of a microbial community growing in an anaerobic digestion system is determined primarily by SRT (Water Pollution Control Federation, 1987). The three reactions (hydrolysis, fermentation, and methanogenesis) are directly related to SRT. There is a minimum SRT for each reaction. If the SRT is less than the minimum SRT, bacteria cannot grow rapidly

enough and the digestion process will fail eventually (WEF, 1998). Typical values for SRT at various temperatures are reported in Table 2.2.

Table 2.2 Suggested solids retention times for use in the design of complete-mix anaerobic digesters (Metcalf & Eddy, 2003).

Operating temperature °C	SRT (minimum)	SRT (design)
18	11	28
24	8	20
30	6	14
35	4	10
40	4	10

In practice for CSTR sludge digestion, values for SRTs range from 10 to 20 days. Grady et al. (1999) observed that a lower SRT limit of 10 days at a temperature of 35°C is sufficient to ensure an adequate safety factor against a washout of the methanogenic population, and incremental changes in volatile solids destruction are relatively small for SRT values above 15 days at 35°C. In selecting the design SRT for anaerobic digestion, peak hydraulic loading must be considered.

2.3.2. Temperature

In anaerobic digestion, temperature is important in determining the rate of digestion, particularly the rates of hydrolysis and methane formation. Maintaining a stable operating temperature is important because the bacteria, especially the methane formers, are sensitive to temperature changes.

Anaerobic digestion is normally carried out within two temperature ranges: mesophilic range (25 – 40 °C) and thermophilic range (higher than 45 °C). Temperature has a positive effect on the digestion rate, resulting in higher volumetric methane production rates. In general, mesophilic anaerobic digestion of sewage sludge is more widely used compared to thermophilic digestion, mainly because of the lower energy requirements and higher stability of the process (Gavala et al., 2003).

2.3.3. pH

The anaerobic digestion system is limited to a relatively narrow pH interval from approx. 6.0 to 8.5; a pH value outside this range can lead to imbalance of the system. Each of the microbial groups involved in anaerobic degradation has a specific pH optimum and can grow in a specific pH range. The methanogens and acetogens have pH optimum at approx. 7, while acidogens have lower pH optima around 6 (Sanchez et al., 2000). Methanogens at pH lower than 6.6 grow very slowly. In most anaerobic digesters, a neutral condition, as indicated by an average pH of 6.8-7.2 is considered normal (Water Pollution Control Federation, 1987).

Horiuchi et al. (2002) suggested that in the anaerobic acid stage reactor the product spectrum of acids strongly depended on the culture pH. Under acidic and neutral conditions the main products were butyric acid, while acetic and propionic acids were the main products under the basic condition. The change in the main products was caused by the change in the dominant microbial population, from butyric acid-producing bacteria to propionic acid-producing bacteria in the acid reactor due to the pH shift. The control of culture pH was considered to be a useful way for controlling the product spectrum in the anaerobic acid reactor.

2.3.4. Alkalinity

With the high CO₂ content in the gas produced in the anaerobic treatment, a well-established, heated digester generally needs a total alkalinity of 2000-2500 mg/l as CaCO₃ to maintain the pH at or near neutral. CO₂ (typical in the range from 30 to 50 percent of the gas produced in the anaerobic treatment) is produced in the fermentation and methnogenesis phases of the digestion process. Due to the partial pressure of gas in a digester, the CO₂ solubilizes and forms carbonic acid, which consumes alkalinity (Water Pollution Control Federation, 1987).

The concentration of alkalinity in a digester is dependent on the solids feed concentration. Alkalinity may vary directly with the raw solids concentration and detention time. Calcium, magnesium, and ammonium bicarbonates are examples of buffering substances found in a digester. Bicarbonate alkalinity is formed in the digester by the reaction of ammonia with carbon dioxide and water to form ammonium bicarbonate. This natural production of alkalinity provides an essential buffer that, in most digestion systems, holds the pH in the desired range of 6.8-7.2 (Metcalf & Eddy, 2003).

pH as a process indicator is strongly dependent on the buffering capacity, or alkalinity, of the system (Ahring et al., 1995). The main buffering species in an anaerobic digester are the VFAs and the bicarbonate. Total alkalinity (TA) measured by titration to a pH endpoint of 4.3, as suggested by APHA (1998), includes both these species. This has been considered as an insensitive parameter for indicating process instability since an increase in VFA concentration will cause a decrease in bicarbonate concentration, resulting in a fairly constant TA-value. The partial alkalinity (PA) as measured by titration to an endpoint pH of 5.75 indicates changes in the bicarbonate concentration. Measuring the PA can be one way of indirectly measuring the VFA accumulation (Bjornsson et al., 2001).

In general, sodium bicarbonate is used for supplementing the alkalinity since it is the only chemical, which shifts the equilibrium to the desired value without disturbing the physical and chemical balance of the fragile microbial population (Rajeshwari et al., 2000).

2.3.5. Volatile acids

The accumulation of VFAs in the acidogenic stage beyond the assimilative capacity of the methanogenic stage may cause instability of the reactor. The inhibiting effect of VFAs will be much higher in systems with low pH, since increased VFA concentration will also cause pH lowering if the alkalinity is low (Kalyuzhnyi, 1997). Typical volatile

acid concentrations are in the range of 50-300 mg/l for a well-digested sludge (Water Pollution Control Federation, 1987).

If the ratio of volatile acids to alkalinity exceeds 0.8, pH depression and inhibition of methane production occurs. Increase above 0.3-0.4 indicates the need for corrective action. A proper ratio for volatile acid to alkalinity is between 0.1-0.2 (Water Pollution Control Federation, 1987).

2.3.6. Organic loading rate

In anaerobic wastewater treatment, organic loading rate plays an important role. An overloaded process is often accompanied by increased acetate concentration. Degradation of propionate and butyrate can be inhibited by high concentrations of acetate, since their degradation rates are thermodynamically limited by the concentration of acetate and hydrogen. Overloading would give rise to increased biogas production in the beginning, followed by a decrease when VFA has accumulated. Gas production is therefore, the earliest and most commonly used parameter for monitoring and control of the anaerobic process (Ahring, 2003).

2.3.7. Nutrient requirement

Some nutritional additions can be necessary to maintain the anaerobic treatment system (Kelly & Switzenbaum, 1984). Nutrients must be available in adequate amounts if a biological system is to function properly. Speece (1996) recommends that nitrogen in the form of NH_4 and phosphorous in the form of PO_4 be provided to stimulate methane production. The required optimum C:N:P ratio for enhanced yield of methane has been reported to be 100:2.5:0.5 (Rajeshwari et al., 2000). Sulfur is also required in the synthesis of proteins and is released in their degradation. As a general rule, for SRT values greater than 7 d, about 5 g nitrogen and 1 g phosphorus will be required per 100 g of BOD to provide an excess of nutrients (Metcalf & Eddy, 2003). Further, to maintain

maximum methanogenic activity, liquid phase concentrations of nitrogen, phosphorus, and sulfur on the order of 50, 10, and 5 mg/l, respectively are desirable.

Some research suggests that the growth of methanogens is also stimulated by vitamins. Jarrell and Kalmokoff (1988) stated that *Methanosaeta soehngeni* requires biotin for growth, while *Methanosarcina barkeri* requires riboflavin and is also stimulated by folic acid. This research also stated that biotin as well as thiamine and PABA are essential for the growth of *Methanoseta concilii*.

2.3.8. Trace nutrients

Trace nutrients, such as iron, are also needed for biological growth. Trace metals play an important role to stimulate methanogenic activity. Selenium, molybdenum, manganese, aluminium, and boron have been recommended as additional components in media (Azbar et al., 2000). The recommended requirements for iron, cobalt, nickel and zinc are 0.02, 0.004, 0.003 and 0.02 mg/g acetate produced, respectively. The exact amounts of trace nutrients needed can vary for different wastewaters. A recommended dose of trace metals per litre of reactor of reactor volume is 1.0 mg FeCl₂, 0.1 mg CoCl₂, 0.1 mg NiCl₂, and 0.1 ZnCl₂ (Metcalf & Eddy, 2003).

2.3.9. Toxicity

A number of compounds are toxic to the anaerobic microorganisms. Methanogens are commonly considered to be the most sensitive to toxicity of the microorganisms in anaerobic digestion.

2.3.9.1. Ammonia

In anaerobic digestion ammonia originates from soluble ammonia in the influent, from protein degradation and other compounds such as urea. There are two forms of ammonia which depend upon the pH of the system: ammonium ions (NH₄⁺), and dissolved non-

ionized form of ammonia (NH_3). It is generally accepted that it is the non-ionized form of ammonia that is responsible for inhibition, pH has a significant effect on the level of ammonia inhibition, as the pH value determine the degree of ionisation.

Ahring et al. (1995) found that the an anaerobic digester could be adapted to tolerate free ammonia concentration of 800 mg-N/l. Velsen (1979) investigated the influence of ammonia-nitrogen concentrations in excess of 1500 mg/l on the methane formation from VFAs by digested sewage sludge, which was acclimated to 815 mg/l ammonia-nitrogen. The methane formation still took place at an ammonia-nitrogen concentration as high as 5000 mg/l. However, an increasing lag-phase was observed at increasing ammonia nitrogen concentrations in the range 730-4990 mg/l. The maximum methane formation rate slowly decreased with increasing ammonia-nitrogen concentrations.

2.3.9.2. Oxygen

Methanogenesis from acetate has generally been considered a strict anaerobic process. Fetzer and Conrad (1993) reported that concentrations of O_2 greater than 0.5% immediately inhibited production of methane by *Methanosarcina barkeri*. The addition of O_2 in concentrations between 0.05-0.1% inhibited methane production after about 0.5 to 2 hours after the O_2 was added. Addition of 0.005% O_2 did not inhibit methane production.

Some researchers conversely suggest that methanogenesis can be enhanced by the presence of small amounts of O_2 . Pirt and Lee (1983) reported that methane production rates were almost doubled by the presence of trace amounts of O_2 . They suggested that methane production should be operated as an oxygen-limited rather than a strictly anaerobic process.

Only small amounts of oxygen might enter the reactors together with strongly aerated substrates in anaerobic digestion processes. Due to the low solubility of oxygen, this does normally not pose a problem to the anaerobic microorganisms in the digester and is rapidly scavenged by facultative bacteria. Kato et al. (1993) demonstrated a high oxygen

tolerance of methanogens in granular sludge due to mainly oxygen consumption by facultatively anaerobic bacteria metabolising easily degradable substrates.

2.3.9.3. Hydrogen

Hydrogen content in the reactor is the key factor for regulating the acidogenesis (Harper and Pohaland, 1986). The symbiosis between hydrogen-producing and hydrogen-consuming microorganisms is confined to a narrow range of hydrogen partial pressures. Propionate, butyrate and higher VFAs require very low hydrogen concentrations for degradation. Propionate degradation requires 5-6 times lower concentrations of hydrogen than butyrate degradation, and accumulation of propionate is often seen during step changes, pulses, and feed composition changes due to short-time increases in hydrogen levels. If the increase in hydrogen is more severe, an increase in butyrate will also be seen (Ahring, 2003).

2.3.9.4. Sulfide

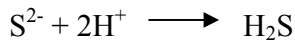
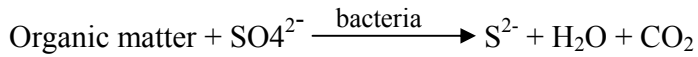
In anaerobic digestion systems, sulfate is reduced biologically under anaerobic conditions to sulfide, which may upset the biological process if the sulfide concentration exceeds 200 mg/l (Metcalf & Eddy, 2003). The concentration of soluble sulphide depends on the pH of the liquid and the composition of the gas space. The effect of soluble sulphide on anaerobic digestion system is shown in table 2.3.

Table 2.3 Effect of soluble sulphide in the anaerobic digestion system. Modified from Grady and Lim (1980).

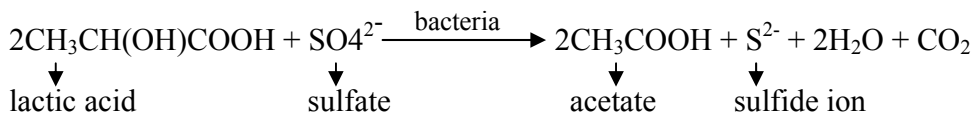
Concentration (mg/l)	Effect
<100	Tolerable, with little or no acclimation
100-200	Little effect after acclimation
>200	Strongly inhibitory

The toxicity of sulfide can be reduced by adding heavy metal ions to the medium since most of them form highly insoluble precipitates with sulphide.

Sulphide can combine with hydrogen to form hydrogen sulfide (H₂S). The following generalized reactions are typical:



If lactic acid is used as the precursor organic compound, the reduction of sulfate to sulfide occurs as follows (Ahring, 2003):



2.4. Anaerobic digestion of primary sludge

Primary sludge is the sludge from primary settling tanks. Primary sludge is usually gray and slimy and, in most cases, has an extremely offensive odor. The typical chemical composition of untreated primary sludge is shown in table 2.4.

Prior to reuse or disposal, primary sludge requires further treatment to reduce pathogen levels and organic content. Primary sludge can be readily anaerobically digested under suitable conditions of operation, particularly the mesophilic single-stage process. As described before, the three types of chemical and biochemical reactions that occur in anaerobic digestion are hydrolysis, fermentation, and methanogenesis.

Hydrolysis of particulate organic matter, the first step in anaerobic degradation, is usually rate-limiting in the overall degradation sequence. In terms of chemical composition, three groups of organic polymers are the major constituents of primary sludge: carbohydrates, proteins and lipids. The particulate carbohydrates are hydrolysed to simple sugars like cellobiose and glucose, which are further degraded to VFAs. Lipids included in sewage

sludge are composed mainly of neutral fats and LCFA. Neutral fats are hydrolysed to LCFA and glycerol. LCFAs are degraded via β -oxidation by H_2 -producing acetogenic bacteria. The occurrence of methanogenic conditions enhances lipids hydrolysis and acidification of LCFA. Proteins are hydrolysed to amino acids that are further fermented to ammonium, VFAs, carbon dioxide, hydrogen gas and reduced sulphur (Mahmoud et al., 2004). Then the final products of fermentation (hydrogen, carbon dioxide, acetate) are degraded further to the final products of methanogenesis (methane and carbon dioxide) by methanogens.

Table 2.4 Typical chemical composition of untreated primary sludge. Adapted, in part, from Metcalf & Eddy (2003).

Item	Range	Typical
Total dry solids (TS), %	5-9	6
Volatile solids (% of TS)	60-80	65
Grease and fats (% of TS)		
Ether soluble	6-30	-
Ether extract	7-35	-
Protein (% of TS)	20-30	25
Nitrogen (N, % of TS)	1.5-4	2.5
Phosphorus (P_2O_5 , % of TS)	0.8-2.8	1.6
Potash (K_2O , % of TS)	0-1	0.4
Cellulose (% of TS)	8-15	10
Iron (not as sulphide, % of TS)	2.0-4.0	2.5
Silica (SiO_2 , % of TS)	15-20	-
pH	5.0-8.0	6.0
Alkalinity (mg/l as $CaCO_3$)	500-1500	600
Organic acids (mg/l as HAc)	200-2000	500
Energy content, kj/kg TSS	23,000-29,000	25,000

2.5. Anaerobic digestion of whey

2.5.1. Definition and characteristics of whey

Whey is a by-product of cheese or casein making from dairy industries. It is the liquid remaining after the recovery of the curds, which are formed by the action of enzymes or acids on milk. There are essentially two types of whey produced depending on the different manufacturing processes. Sweet whey has a pH value greater than 5.5 and is derived from the manufacture of cheese or rennet casein. Manufacture of cottage cheese, lactic casein or mineral acid casein produces acid whey with a pH value less than 5 (Short, 1978).

Whey comprises 80-90% of the total volume of milk entering the process and contains more than half the solids present in the original whole milk, including 20% of the protein and most of the lactose and most of the water soluble vitamins and minerals. Whey has a high organic content with a chemical oxygen demand (COD) of about 60-80 g/l (Patel & Madamwar, 1998).

Table 2.5 summarizes the approximate composition of whey. The major components are lactose, proteins and minerals. The protein fraction comprises about 90% α -lactalbumin and β -lactoglobulin which have a high nutritional value due to their favourable amino acid composition and especially their high lysine content (Short & Doughty, 1977).

Table 2.5 Approximate composition of whey, Bylund (1995)

Constituent	Cheese whey (%)	Casein whey (%)
Total solids	6.4	6.5
Water	93.6	93.5
Fat	0.05	0.04
True protein	0.55	0.55
NPN (non-protein nitrogen)	0.18	0.18
Lactose	4.8	4.9
Ash (minerals)	0.5	0.8
Calcium	0.043	0.12
Phosphorus	0.040	0.065
Sodium	0.050	0.050
Potassium	0.16	0.16
Chloride	0.11	0.11
Lactic acid	0.05	0.4

2.5.2. Disposal problem of whey

Each ton of cheese and casein manufactured produces approximately 7.6 and 25 tons of whey, respectively. Therefore, large volumes of whey are produced, for example approximately 2.5 million tons of whey (based on cheese and casein production) were produced in New Zealand in 1989 (New Zealand Dairy Board 1989). Disposal of whey makes up a significant part of environmental problem and contribute substantially to the pollution of surface waters and soil (Kosseva et al., 2003). While whey may have further uses (such as protein and lactose recovery), some small and medium-size dairy industries do not have the economic incentive to do so, making it necessary to consider its treatment as a waste stream (Mockaitis et al., 2005).

2.5.3. Factors that affect the efficiency of anaerobic digestion of whey

Because of the high organic content of whey, anaerobic digestion is essentially the only viable treatment method (Gavala et al., 1999). However, whey has very high biodegradability (about 99%) and low bicarbonate alkalinity, therefore whey tends to acidify rapidly which accumulate VFAs. This may cause instability of the system as the accumulation of VFAs in the acidogenic stage beyond the assimilative capacity of the methanogenic stage. According to Schroder and De Haast (1989) a highly biodegradable carbon source, such as cheese whey, would cause inhibition to occur due to imbalance in the rates of acidogenesis and methanogenesis.

The required pH range for methanogenesis is between 6.6 and 7.6 with an optimum range between 7.0 and 7.2 (Ghaly, 1996). The treatment of whey by anaerobic degradation is constrained by the drop in pH due to the accumulation of VFAs that inhibits further conversion of acids to methane. Anaerobic digestion of acid cheese whey without pH control is not feasible (Ghaly, 1996).

Supplemental alkalinity is required so as to avoid anaerobic process failure (Mockaitis et al., 2005). Marshal and Timbers (1982) reported that a 500 l pilot-scale fixed-film reactor receiving raw whey needed the addition of NaOH for pH control. Norstedt and Thomas (1984) found that without pH control, an anaerobic fixed-bed reactor could not achieve stable operation within 30 days.

The major component of whey is lactose, which is quickly fermented to VFAs and hydrogen (Ahring, 2003). The symbiosis between hydrogen-producing and hydrogen-consuming microorganisms is confined to a narrow range of hydrogen partial pressures. Reactions become thermodynamically unfavorable for one or the other part of the relationship if outside the hydrogen partial pressures. This can be caused by overloading with easily degradable compounds or by unintentional influence of inhibitory compounds (Scheper and Ahring, 2003).

Methanogenesis is the rate-limiting step of the overall process (Valentini et al., 1997). In addition, some nutritional additions were thought to be necessary to maintain the system stability (Kelly & Swizenbaum, 1984).

2.5.4. Previous studies

Yilmazer and Yenigun (1999) investigated the performance of two-phase anaerobic digestion of cheese whey in a system consisting of a continuous stirred tank reactor (CSTR) as the acidogenic reactor and an upflow anaerobic filter (UFAF) as the methanogenic reactor. They suggested that an optimum HRT for the acidogenic reactor with the same organic loading rate (OLR) between 0.5-2 g COD/MLSS day was 24 hours. For the methanogenic reactor, at an HRT of 4 days a 90% soluble effluent COD removal efficiency was obtained with a biogas yield of 0.55 m³/kg COD removed. It was necessary to add some micro and macro nutrients to the cheese whey feed solution. Ammonium bicarbonate and dipotassium hydrogen phosphate were added to adjust the COD/N/P ratio to 250:5:1 (Yilmazer & Yenigun, 1999). The concentrations of the micro and macro nutrients added to the cheese whey feed solution are given in Table 2.6 (for COD concentration of 20,000 mg/l).

Table 2.6 Nutrient solution composition used by Yilmazer and Yenigun (1999)

Macronutrient	Concentration	Micronutrient	Concentration
K ₂ HPO ₄	448 mg/l	FeCl ₃ .6H ₂ O	4.830 mg/l
NH ₄ HCO ₃	2256 mg/l	NiSO ₄ .6H ₂ O	0.444 mg/l
		CoCl ₂ .6H ₂ O	0.402 mg/l
		(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	0.036 mg/l

Ince et al. (1997) noted that the treatment capacity of an anaerobic digestion system is primarily determined by the amount of methanogenic population retained within the system. They reported that the COD:N:P ratio in the influent was maintained at a ratio of 400:5:1 by adding urea and KH₂PO₄ in order to supplement nitrogen and phosphorus. The pH of the feed was adjusted to neutral during the start-up period. They suggested that

an acclimatization period was necessary for the biomass in the digester for the new wastewater and the different reactor configuration. Acclimatization of the digester sludge was completed after 40 days operation after which the COD removal efficiency of the system was found to be over 98%.

Mockaitis et al. (2005) made an assessment of cheese whey treatment in a mechanically stirred anaerobic sequencing batch reactor (ASBR). They investigated the effect of increasing organic load and decreasing influent supplemented alkalinity (as sodium bicarbonate) on process efficiency and stability. The reactor was operated with increasing influent whey concentrations of 500, 1000, 2000 and 4000 mgCOD/l. For each condition a startup procedure was adopted, in which operation started with low whey concentration (about 500 mgCOD/l), for sludge acclimatization, and the medium was supplemented with alkalinity. This supplementation started with a mgNaHCO₃/mgCOD ratio of 1:1 in the influent and was reduced as the system stabilized, to optimise the amount effectively required to confer operation stability. The optimum alkalinity supplementation was 50% in the assays with 500 and 1000 mgCOD/l and 25% in the assays with 2000 and 4000 mgCOD/l. alkalinity supplementation to the system enabled an increase in influent organic load. Increasing organic load promotes increased accumulation of volatile acids and decreased methane production likely due to inhibition of the anaerobic process by the accumulation of volatile acids.

Anaerobic digestion of whey in different reactor configurations has been reported in Table 2.7.

Table 2.7 Comparison of various anaerobic treatment processes for whey.

Reactor type ^a	HRT (days)	Influent concentration (g COD/l)	OLR (g COD/l.d)	Removal efficiency (%)	Reference
UFFLR	5	79	14	95	Wildenauer & Winter, 1985
DSFFR	5	13	2.6	88	De Haast et al., 1985
FBR	0.4	7	7.7	90	Boening & Larsen, 1982
FBR	0.1-0.4	0.8-1.0	6-40	63-87	Denac & Dunn, 1988
AAFEB	0.4-1.1	10	8.9-27	77-93	Switzenbaum & Danskin, 1982.
AnRBC	0.6-0.7	5-15	8.2-22	61-92	
AnRBC	5	64	10.2	76	Lo & Liao, 1986
	6-11	61-70	6.3-10	76	
AnRBC	1-5	30	6-30	65-80	Patel & Madamwar, 1998
SDFR		69.8	16.1	99	Barford et al., 1986
UASB	1.5	11	7.1	94	Schorder & De Haast, (1998)
UASB	5	5-28.7	0.9-6	97-99	Yan et al., 1989
UASB	2.3-11.6	5-77	1-28.5	95-99	Kalyuzhnyi et al., 1997
	5.4-6.8	47-55	7-9.5	90-94	
UASB	2.06-4.95	43-58	11.7-24.6	91.9-97.0	Erguder et al., 2001
DUHR	7	68	10	97	Malaspina et al., 1995
TSUAD	10	69.6	7	32.5	Ghaly, 1996
	20	69.6	3.5	39.5	
ASBR	0.33	0.5-4	0.6-4.8	90 (filtered samples)	Mockaitis et al., 2005

^aUFFLR = upflow fixed-film loop reactor

DSFFR = downflow stationary fixed-bed reactor

FBR = fluidised-bed reactor

AAFEB = anaerobic attached-film expanded-bed reactor

AnRBC = anaerobic rotating biological contact reactor

SDFR = semicontinuous digester with flocculant addition

UASB = upflow anaerobic sludge-blanket reactor

DUHR = downflow-upflow hybrid reactor

TSUAD = two-stage unmixed anaerobic digester

ASBR = anaerobic sequencing batch reactor

Little study on anaerobic co-digestion of municipal primary sludge and whey has been done. Lo et al. (1988) investigated the anaerobic co-digestion of a mixture of cheese whey and dairy manure. They reported that the AnRBC could be operated at a HRT as low as 2 days without the need to add any buffering reagent to the mixture. The stability of the AnRBC receiving a mixture of cheese whey and dairy manure was improved, as indicated by the fact that the AnRBC receiving cheese whey alone could not be operated at HRTs below 5 days, while that receiving the mixture was operated at a HRT as low as 2 days. They explained that the dairy manure provided the nutrient supplement and buffering capacity, therefore, eliminated the need for pH control and addition of nutrients.

CHAPTER 3 MATERIALS AND METHODS

3.1. Materials

The digester sludge and primary sludge used in the experiments were supplied by the wastewater treatment plant of Palmerston North City Council, New Zealand. The digester sludge collected from the full-scale anaerobic digester of the wastewater treatment plant was used as inoculum of the reactors. The primary sludge used as substrate was collected once a week. The primary sludge was stored in a refrigerator at 5°C and was warmed up to about 35°C by putting into constant temperature room with temperature of 35°C for 4 hours prior to use. The COD of the primary sludge ranged from 62 to 90 g COD/l.

Whey powder was used as co-substrate in the experiments because the consistent characteristics of whey powder. Moreover, using powder eliminated the HRT issues in the experiments allowing focus on organic loading rate variation without changing HRT. The COD of the whey powder was approximately 1.9 g COD/g whey powder.

3.2. Analytical procedures

3.2.1. Chemical Oxygen Demand (COD)

The Chemical Oxygen Demand (COD) was determined using the standard method 5220-chemical oxygen demand (COD) D (closed reflux, colorimetric method) (APHA, 1998). The method is based on the fact that most types of organic material are oxidized by a boiling mixture of digestion solution ($K_2Cr_2O_7$, H_2SO_4 and $HgSO_4$) and sulphuric acid reagent (H_2SO_4 containing Ag_2SO_4 as catalyst).

For analysing, 2.5 ml liquid sample, 1.5 ml digestion solution and 3.5 ml sulphuric acid reagent were well mixed and heated in a COD reactor (HACH) at 150°C for 2 hours. After oxidation, the mixture was measured by a spectrophotometer, using a wavelength

of 600 nm. The absorbance of the oxidation result was converted to COD concentration by comparing with a standard curve of KHP (potassium hydrogen phthalate).

3.2.2. Total Kjeldahl Nitrogen (TKN)

Total Kjeldahl Nitrogen (TKN) was determined by using Method 4500-Norg B Macro-Kjeldahl Method of Standard Methods (APHA, 1998). Kjeldahl nitrogen is the sum of organic nitrogen and ammonia nitrogen. Most of organic compounds containing nitrogen were derivatives of ammonia and destruction of organic portion of the molecule released the nitrogen as ammonia. The Kjeldahl method employed sulphuric acid as the oxidizing agent. A catalyst was needed to hasten the oxidation of some the more resistant organic substances. The oxidation proceeded rapidly at temperatures slightly above the boiling point of sulphuric acid (340°C). The boiling point of the acid was increased by addition of sodium or potassium sulphate. When the organic nitrogen has been released as ammonia nitrogen, it was measured by using Method 4500-NH₃ B of APHA (1998) with preceding distillation.

3.2.3. Solid Content (Total Solid-TS and Volatile Solid-VS)

The solid content was determined by using Standard Method 2540 Solids (APHA, 1998). The well mixed samples were measured in a clean evaporating crucible (known weight). Then the samples were evaporated and dried to constant weight. For determining the total solid (TS), the sample was evaporated at 103 to 105°C. The volatile solid (VS) was obtained by subtraction of the mineral content (residual ash after igniting at 550°C) from the total solid content.

3.2.4. Other analysis

The VFA and lactate concentrations were measured using ion chromatography (Dionex ICS-2000, using an IonPac AS11-HC column). The biogas produced during anaerobic digestion was measured by gas meters. Gas composition was measured by gas

chromatograph (Shimadzu, GC-8A). Ammonia was determined by using Method 4500-NH₃ B of Standard Method (APHA, 1998). Ammonia in the sample was distilled into a solution of boric acid and determined titrimetrically with standard 0.1 M HCL with mixed indicator. The pH value of samples was measured using a portable pH meter (Model 230A, supplied by WATSON VICTOR LTD, New Zealand). Before measurement the pH meter was calibrated with standard buffer solutions at pH 4.0 and 7.0. The phosphate was measured using ion chromatography (Dionex ICS-2000, using an IonPac AS11-HC column). A GBC 933 Atomic Absorption (AA) Spectrophotometer (supplied by GBC Scientific Equipment Pty Ltd, Melbourne, Australia) was used to measure the metal concentrations of the samples. Alkalinity was measured by using Standard Method 2320 alkalinity (APHA, 1998).

3.3. Experiments

3.3.1. Semi-continuous whey digestion with pH control

The purpose of this experiment was to investigate the performance of anaerobic semi-continuous whey digestion with pH control.

An anaerobic semi-continuous stirred tank reactor (radius = 0.1m, height = 0.5 m) was set up in a constant temperature incubator with temperature of 35°C. A photo graph of the reactor is shown in Figure 3.1. Digester sludge (5 l) was seeded in the reactor. A pH electrode was inserted in the reactor and was connected to pH meter-controller controlling a peristaltic pump in on/off mode. When the pH value was below 6.8, the pump was turned on. The pump transferred 1.8 M sodium hydroxide solution to the reactor. When the pH value reached 7.2, the pump was switched off. The reactor was sealed by silicon grease to avoid oxygen.

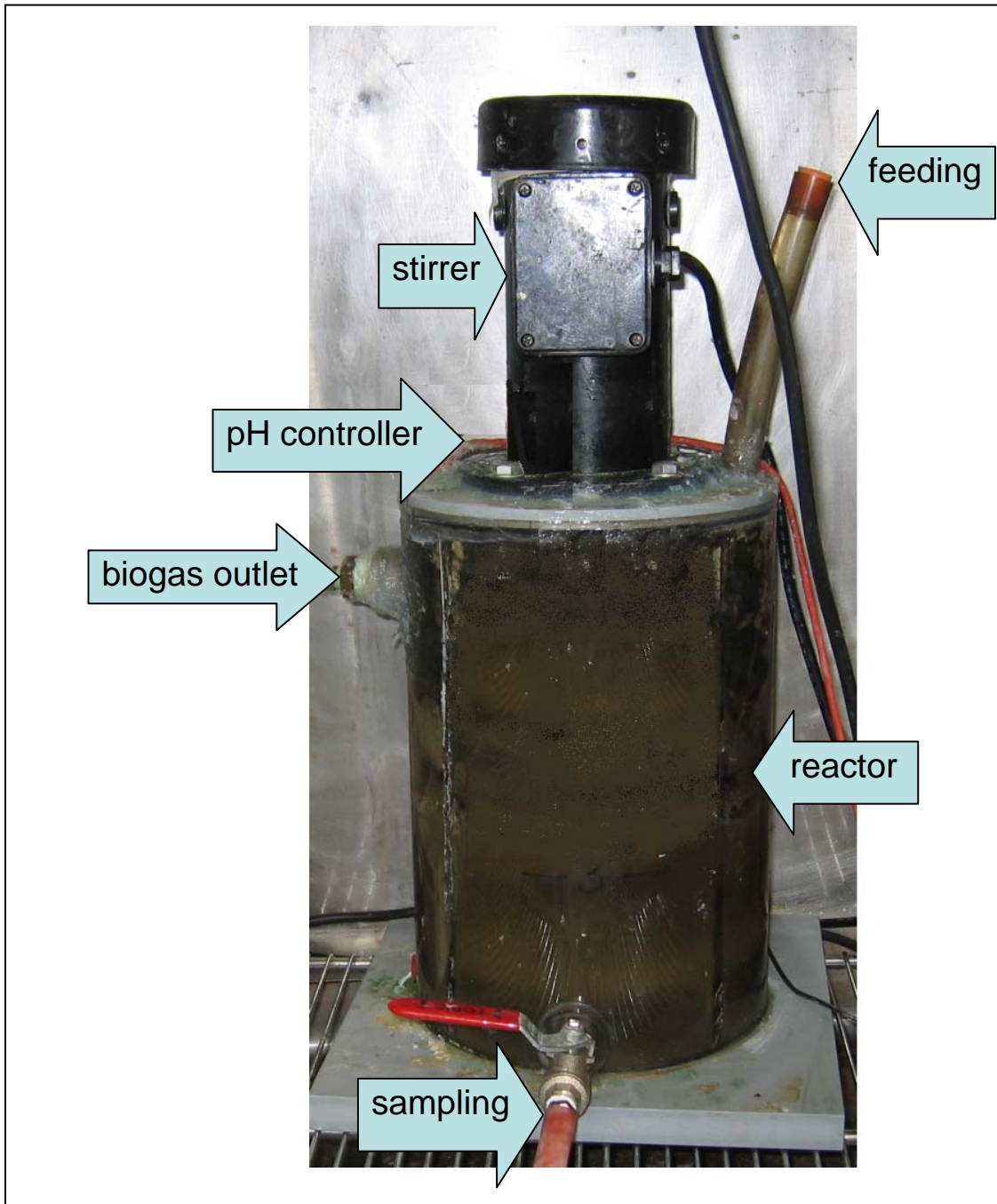


Figure 3.1 Scheme of the anaerobic semi-continuous stirred tank reactor used in this study

Known weight of whey powder was fed daily and was mixed thoroughly with the digester sludge in the reactor during the first 34 days. In order to investigate the effect of organic loading rate (OLR) on the performance of the reactor, the OLR was adjusted to 1

g/l.d from day 1 to day 18 and 3 g/l.d from day 19 to day 34. No whey powder was fed into the reactor after day 34 due to the poor performance of the reactor (this will be discussed in 4.1.1). In order to improve the performance of the reactor and investigate the effect of nutrient addition on the performance of the reactor, nutrients ((NH₄)₂(CO₃).H₂O 6g, KH₂PO₄ 0.3g, K₂HPO₄ 0.45g) were added at day 39, 40 and 41 to adjust the C/N/P ratio to 100:2.5:0.5. Furthermore, in order to investigate the effect of trace element addition on the performance of the reactor, 45 ml trace element solution was added at day 131, 27 mg ZnSO₄.7H₂O was added at day 151, and 76 mg FeCl₃.6H₂O was added at day 156, as shown in Table 3.1. 10 ml sludge was taken out daily from the sampling point at the bottom of the reactor for sampling purpose.

TCOD and SCOD in the reactor were measured daily. VFAs, TS and VS were measured once a week.

Table 3.1 Substrate added in the reactor of the semi-continuous experiments

Time (d)	1-18	19-34	39-41	131	151	156
Whey powder (g/d)	2.7	8.0				
^a Nutrients (set/d)			1			
^b Trace element solution (ml)				45		
ZnSO ₄ .7H ₂ O (mg)					27 (=6.2 mg Zn)	
FeCl ₃ .6H ₂ O (mg/d)						76 (= 15.8 mg Fe)

^a One set nutrients contained: (NH₄)₂(CO₃).H₂O 6g, KH₂PO₄ 0.3g, K₂HPO₄ 0.45g.

^b One litre trace element solution contained: FeCl₃.6H₂O 1.5g, H₃BO₃ 0.15g, CuSO₄.5H₂O 0.03g, KI 0.03g, MnCl₂.4H₂O 0.12g, Na₂MoO₂.2H₂O 0.06 g, ZnSO₄.7H₂O 0.12g, and CoCl₂.6H₂O 0.15g.

3.3.2. Co-digestion of primary municipal sludge and whey

The main objectives of the co-digestion of primary sludge and whey study were as follows:

- To investigate the feasibility and the performance of co-digestion of primary sludge and whey without addition of nutrients, trace elements and metals.
- To investigate the factors affecting the performance of the co-digestion reactors.
- To investigate the OLR of co-digestion of primary sludge and whey.

The anaerobic continuous stirred reactors used in the co-digestion experiments were set up in a constant temperature room with temperature of 35°C. The scheme of the anaerobic continuous stirred reactors used in this study is shown in Figure 3.2. Each reactor was a 2.5 litre Buckner flask and was stirred with a magnetic stirrer and a stir bar. Each reactor was inoculated with 2 litre digester sludge taken from the anaerobic digester of Palmerston North City Council wastewater treatment plant. 100 ml effluent was withdrawn daily from the overflow port at the side of each reactor, and 100 ml substrate was fed to each reactor daily from the top of the reactor. After feeding, each reactor was covered by the lid and was sealed by silicon grease to avoid leaking. Biogas left the reactor through a port at the top and was measured with a wet gas meter.

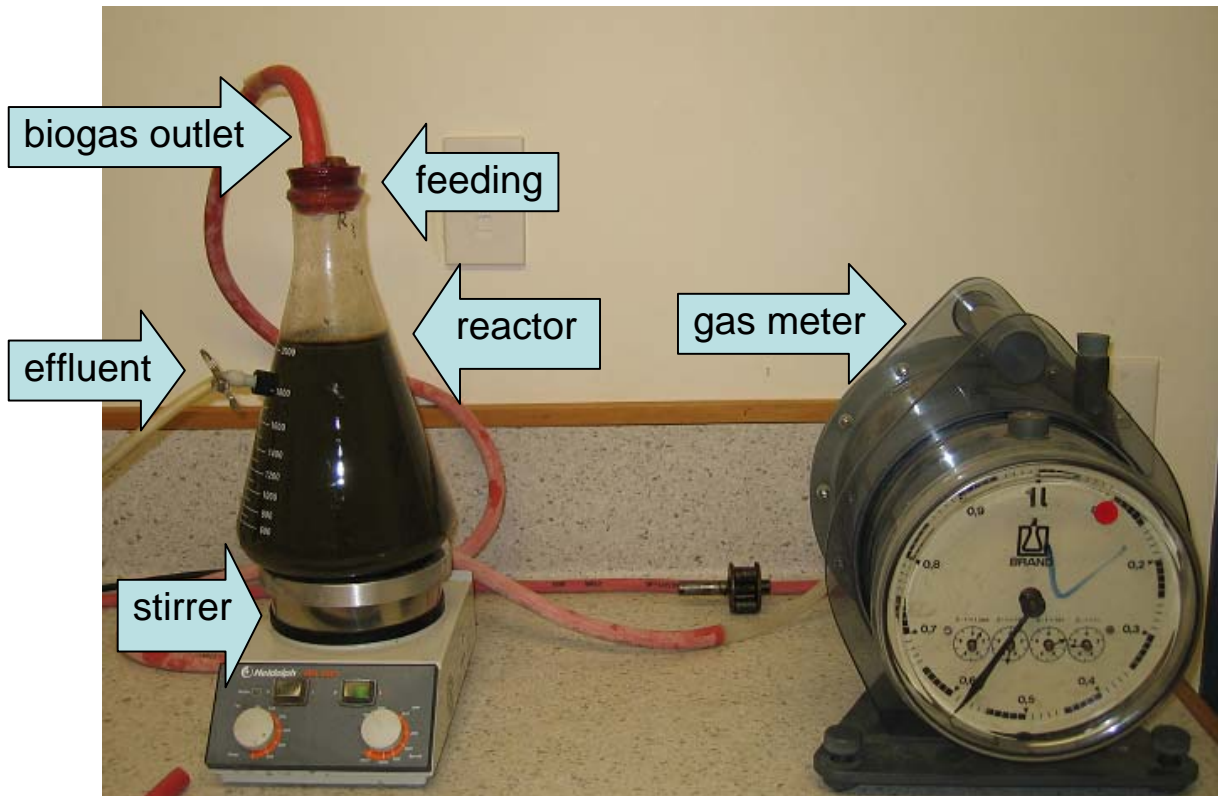


Figure 3.2 Scheme of the anaerobic continuous stirred reactor used in this study

3.3.2.1. Investigation of the effect of COD loading ratio of primary sludge to whey on the performance of the reactors

The purpose of this experiment was to investigate the feasibility of co-digestion of primary sludge and whey as well as the effect of the COD loading ratio of primary sludge to whey on the reactor performance.

Three anaerobic continuous stirred reactors (namely, reactor A, reactor B and reactor C) were set up. 100 ml effluent was withdrawn daily from the overflow port at the side of each reactor, and 100 ml substrate was fed to each reactor daily from the top of the reactor. The HRT of the three reactors were 20 days.

Reactor A was fed with 100 ml primary sludge as a control, while reactor B and reactor C were fed with different volume of primary sludge and whey solution (66 ± 2 g COD/l),

prepared from whey powder and Milli-Q water, with total volume of 100 ml. The volume of primary sludge (PS) and whey solution fed in the three reactors are shown in Table 3.2. The COD loading ratio of primary sludge to whey and the OLR of each reactor are also shown in Table 3.2. Due to the COD variation of the primary sludge (from day 1 to 7, 66 ± 2 g COD/l; from day 8, 80 ± 2 g COD/l), the OLR of the three reactors were various from day 8.

The performances of the three reactors were monitored by measuring the pH, TCOD, SCOD, bicarbonate alkalinity, VFA and lactate concentrations of the effluent.

Table 3.2 Feed comparison of reactor A, reactor B and reactor C

Time (d)	Reactor	HRT (d)	Feed		OLR (g COD/l.d)	COD loading ratio (PS : whey)	pH control
			PS (ml)	whey solution (ml) (66 ± 2 g COD/l)			
Day 1-7	A	20	100	0	3.3 ± 0.1	100:0	No
	B	20	70	30	3.3 ± 0.1	70:30	No
	C	20	30	70	3.3 ± 0.1	30:70	No
Day 8-30	A	20	100	0	4.0 ± 0.1	100:0	No
	B	20	70	30	3.8 ± 0.1	69:31	No
	C	20	30	70	3.5 ± 0.1	33:67	No

3.3.2.2. Investigation of the effect of pH on co-digestion of primary sludge and whey

In order to investigate the effect of pH on co-digestion of primary sludge and whey, after 64 days of operation without pH control, reactor C which pH dropped to 6.1 (will discuss in 4.2.1.2) was changed to reactor C' in that the pH was maintained between 6.8 and 7.0 by adding sodium bicarbonate (NaHCO_3) (called pH control in this study). Other feeding conditions remained same as reactor C, as shown in Table 3.3.

In order to redo reactor C and reactor C' at higher OLR, another two continuous stirred reactors were set up (namely, reactor D and reactor D'), both reactors with HRT of 20 days. Reactor D was fed with 43 ml primary sludge and 57 ml whey solution (145 ± 2 g COD/l) per day without pH control. Reactor D' was fed with similar substrate to reactor D (43 ml primary sludge and 57 ml whey solution per day), but with pH control ($\text{pH} = 6.9 \pm 0.1$). The OLR and the COD loading ratio of primary sludge to whey of the two reactors are shown in Table 3.3.

The pH, TCOD, SCOD, bicarbonate alkalinity, VFA and lactate concentrations of the effluent of the reactors were measured.

Table 3.3 Feed comparison of reactor C, reactor C', reactor D and reactor D'

Reactor	HRT (d)	Feed		COD loading ratio (PS : whey)	OLR (g COD/l.d)	pH control
		PS (ml)	whey solution (ml)			
C	20	30	70 (66 ± 2 g COD/l)	33:67	3.5 ± 0.1	No
C'	20	30	70 (66 ± 2 g COD/l)	33:67	3.5 ± 0.1	Yes (6.9 ± 0.1)
D	20	43	57 (145 ± 2 g COD/l)	30:70	5.8 ± 0.1	No
D'	20	43	57 (145 ± 2 g COD/l)	30:70	5.8 ± 0.1	Yes (6.9 ± 0.1)

3.3.2.3. Investigation of OLR for co-digestion of primary sludge and whey without pH control

In order to increase OLR for co-digestion of primary sludge and whey without pH control, reactor F was set up. Reactor F was fed with 100 ml primary sludge and 1.9 g whey powder per day without pH control. The OLR was increased by adding additional whey powder without changing the feeding volume of 100 ml primary sludge. Therefore, the

HRT of reactor F was 20 days. The amount of primary sludge (PS) and whey powder fed in reactor F are shown in Table 3.4. The OLR and the COD loading ratio of primary sludge to whey of the reactor are also shown in Table 3.4. The pH, TCOD, SCOD, bicarbonate alkalinity, VFA and lactate concentrations of the effluent were measured.

Table 3.4 The amount of primary sludge (PS) and whey powder fed in reactor F

Reactor	HRT (d)	Feed		COD loading ratio (PS : whey)	OLR (g COD/l.d)	pH control
		PS (ml)	whey powder (g)			
F	20	100	1.9	67 : 33	5.8 ± 0.1	No

3.3.2.4 Increasing OLR for co-digestion of primary sludge and whey with pH control

In order to determine what further increase in OLR for co-digestion of primary sludge and whey could be achieved with pH control, other three reactors (namely reactor G, reactor H and reactor I) were set up. The OLR was increased by adding additional whey powder without changing the feeding volume of 100 ml primary sludge. The HRT of the three reactors were 20 days. The amount of primary sludge (PS) and whey powder fed in the reactors are shown in Table 3.5. The OLR and the COD loading ratio of primary sludge to whey of the reactors are also shown in Table 3.5.

Table 3.5 Feed comparison of reactor G, reactor H and reactor I

Reactor	HRT (d)	Feed		COD loading ratio (PS : whey)	OLR (g COD/l.d)	pH control
		PS (ml)	whey powder(g)			
G	20	100	3.8	54 : 46	7.6 ± 0.1	Yes (6.9 ± 0.1)
H	20	100	4.4	48 : 52	8.1 ± 0.1	Yes (6.9 ± 0.1)
I	20	100	5.5	44 : 56	9.4 ± 0.1	Yes (6.9 ± 0.1)

The pH, TCOD, SCOD, bicarbonate alkalinity and VFA concentrations of the effluent were measured.

3.3.2.5. Investigation of the effect of OLR on co-digestion of primary sludge and whey with different HRT

In previous co-digestion of primary sludge and whey experiments (section 3.3.2.3 and 3.3.2.4), the OLR was increased by adding more whey powder, so that the HRT of each reactor remained 20 days. However, in reality whey is presented as liquid. In order to increase OLR using primary sludge and whey solution as substrate to simulate the realistic situation, decreasing HRT was necessary. The purpose of this experiment was to investigate the effect of OLR on co-digestion of primary sludge and whey with different HRT.

Reactor J was set up treating 100 ml primary sludge and whey solution (66 ± 2 g COD/l) which increased by 2 ml each day gradually from 2 ml to 82 ml during the first 40 days of co-digestion without pH control. This resulted in the OLR increasing from 3.2 to 7.1 g COD/l.d, the HRT decreasing from 20 days to 11 days, and the COD loading ratio of primary sludge to whey decreasing from 98 : 2 to 61 : 39, as shown in Table 3.6.

Table 3.6 The amount of primary sludge (PS) and whey solution fed in reactor J

Reactor	HRT (d)	Feed		COD loading ratio (PS : whey)	OLR (g COD/l.d)	pH control
		PS (ml)	Whey solution (ml)			
J	20~11	100	2~82	98~61 : 2~39	3.2~7.1	No

The pH, TCOD, SCOD, bicarbonate alkalinity, VFA and lactate concentrations of the effluent of reactor J were measured.

3.3.2.6. Investigation of the effect of HRT on co-digestion of primary sludge and whey with constant OLR

The purpose of the experiment was to investigate of the effect of HRT on co-digestion of primary sludge and whey with constant OLR (7.6 ± 0.1 g COD/l.d, same as reactor G).

Comparing to reactor G (HRT = 20 days, see 3.3.2.4), which treated 100 ml primary sludge and 3.8 g whey powder per day with pH control ($\text{pH} = 6.9 \pm 0.1$), another reactor (reactor K, HRT = 10 days) was set up, which treated 100 ml primary sludge and 3.8 g whey powder dissolved in 100 ml Milli-Q water per day with pH control ($\text{pH} = 6.9 \pm 0.1$). Table 3.7 shows the feed comparison of reactor G and reactor K.

Table 3.7 Feed comparison of reactor G and reactor K

Reactor	HRT (d)	Feed			COD loading ratio (PS : whey)	OLR (g COD/l.d)	pH control
		PS (ml)	water (ml)	whey (g)			
G	20	100	0	3.8	53 : 47	7.6 ± 0.1	Yes (6.9 ± 0.1)
K	10	100	100	3.8	53 : 47	7.6 ± 0.1	Yes (6.9 ± 0.1)

The pH, TCOD, SCOD, bicarbonate alkalinity, VFA and lactate concentrations of the effluent of the reactors were measured.

CHAPTER 4 RESULTS AND DISCUSSION

4.1. Semi-continuous whey digestion with pH control

The purpose of this experiment was to investigate the performance of anaerobic semi-continuous whey digestion with pH control.

An anaerobic semi-continuous stirred tank reactor (radius = 0.1m, height = 0.5 m) was set up in a constant temperature incubator with temperature of 35°C. Digester sludge (5 l) was seeded in the reactor. The pH value of the reactor was controlled between 6.8 and 7.2.

Known weight of whey powder was fed and was mixed thoroughly with the digester sludge daily in the reactor during the first 34 days. OLR was 1 g/l.d from day 1 to day 18 and 3 g/l.d from day 19 to day 34. TCOD and SCOD in the reactor were measured daily. VFAs, TS (Total Solids) and VS (Volatile Solids) were measured once a week.

TCOD, SCOD and SCOD removal efficiency in the reactor during the first 35 days of the experiment are shown in Figure 4.1.

As can be seen from Figure 4.1, the initial SCOD in the reactor was about 2348 mg/l. During the first 18 days, the OLR was 1 g COD/l.d. After 1 day of reaction, the SCOD in the reactor decreased to 0, which gave a SCOD removal efficiency of 100%. From day 2 to day 3, the SCOD removal efficiency decreased to 89%. From day 3 to day 11, the SCOD removal efficiency remained between 81% and 72%. After that, the SCOD removal efficiency began to decrease from 75.6% at day 11 to 16.2% at day 18 as the TCOD and SCOD began to accumulate. In order to investigate the effect of OLR on the SCOD removal efficiency, the OLR increased to 3 g COD/l.d from day 19 to day 34. However, the SCOD removal efficiency kept decreasing sharply from 16.2% to 2.6%. The SCOD accumulated from 2803 to 37424 mg/l and the TCOD accumulated from 26136 to 63030 mg/l. This indicated that the reactor was failing at OLR of 3 g COD/l.d. Therefore, no whey was fed after day 34.

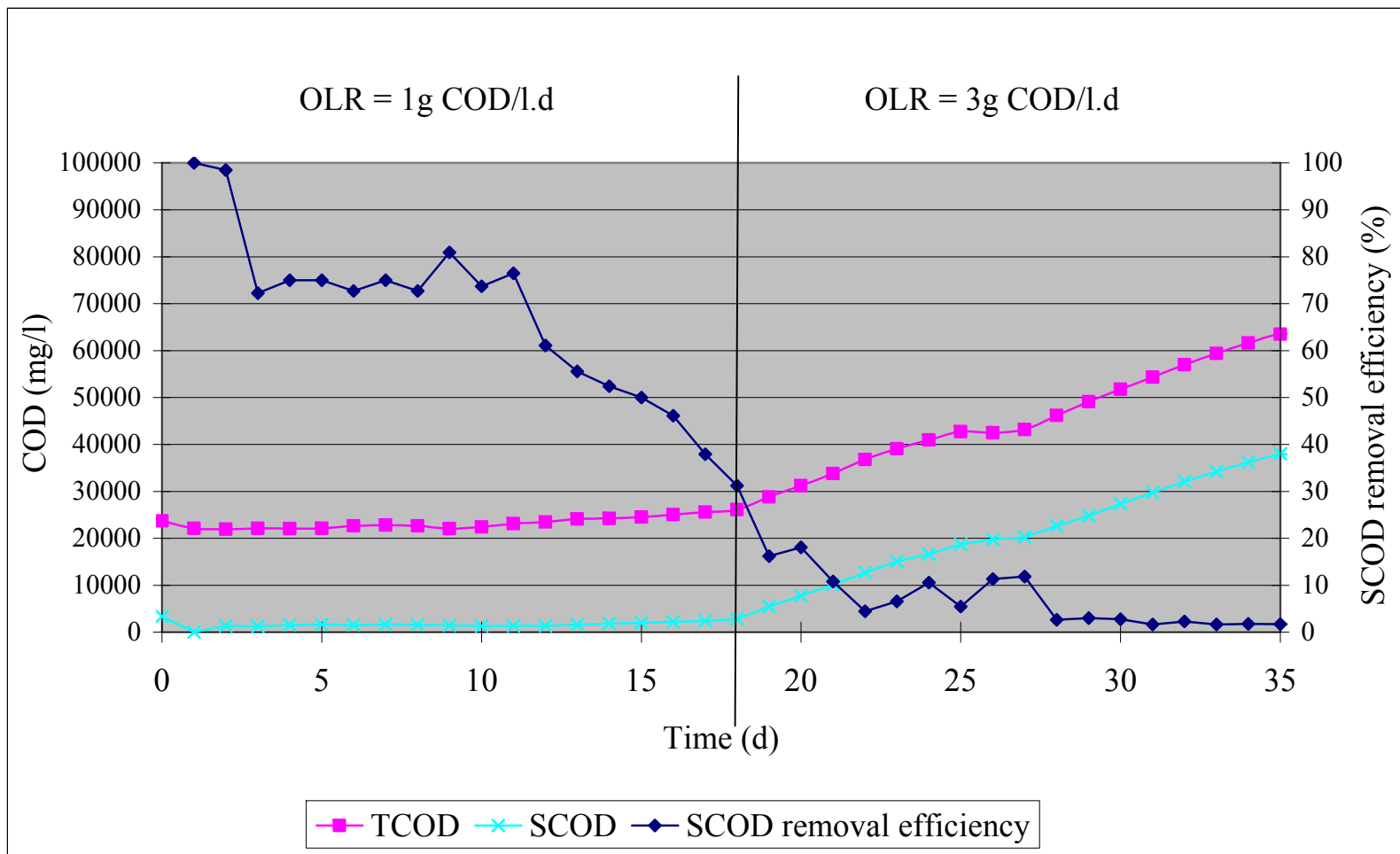


Figure 4.1 TCOD, SCOD and SCOD removal efficiency in the reactor of the semi-continuous experiment (day 0 to day 35).

4.1.1. Effect of nutrient addition on COD removal

The accumulation of TCOD and SCOD was suspected to be caused by insufficient nutrients in the substrate. According to Rajeshwari et al. (2000), the required optimum C:N:P ratio for anaerobic digestion should be 100:2.5:0.5. However, in this study the C:N:P ratio of whey powder was 100:0.1:0.3, which indicated that the nitrogen and phosphorus were deficient.

In order to investigate the effect of nutrient addition on the performance of the reactor, nutrients ((NH₄)₂(CO₃).H₂O 6g, KH₂PO₄ 0.3g, K₂HPO₄ 0.45g) were added at day 39, 40 and 41 to adjust the C/N/P ratio to around 100:2.5:0.5. TCOD, SCOD and SCOD removal efficiency in the reactor during the first 45 days of the experiment are shown in Figure 4.2.

Figure 4.2 shows that after addition of nutrients, the SCOD and TCOD decreased significantly. SCOD decreased from 35758 mg/l to 28182 mg/l, and TCOD decreased from 61667 mg/l to 55000 mg/l. The SCOD removal efficiency increased from 2.6% to 9.7% at day 40, 7.5% at day 41, and 5.6% at day 42 when nutrients were added.

The results of the experiment suggested that addition of nutrients increased SCOD removal efficiency because the elements nitrogen and phosphorus were essential to the growth of microorganisms. This was in agreement with Kelly and Switzenbaum (1984) who suggested that nutrients must be available in adequate amounts if a biological system is to function properly. Speece (1996) recommended that nitrogen in the form of NH₄ and phosphorous in the form of PO₄ should be provided to stimulate anaerobic digestion. The required optimum C:N:P ratio for enhanced yield of methane has been reported to be 100:2.5:0.5 (Rajeshwari et al., 2000).

Yilmazer and Yenigun (1999) investigated the performance of two-phase anaerobic digestion of cheese whey in a system consisting of a continuous stirred tank reactor (CSTR) as the acidogenic reactor and an upflow anaerobic filter (UFAF) as the

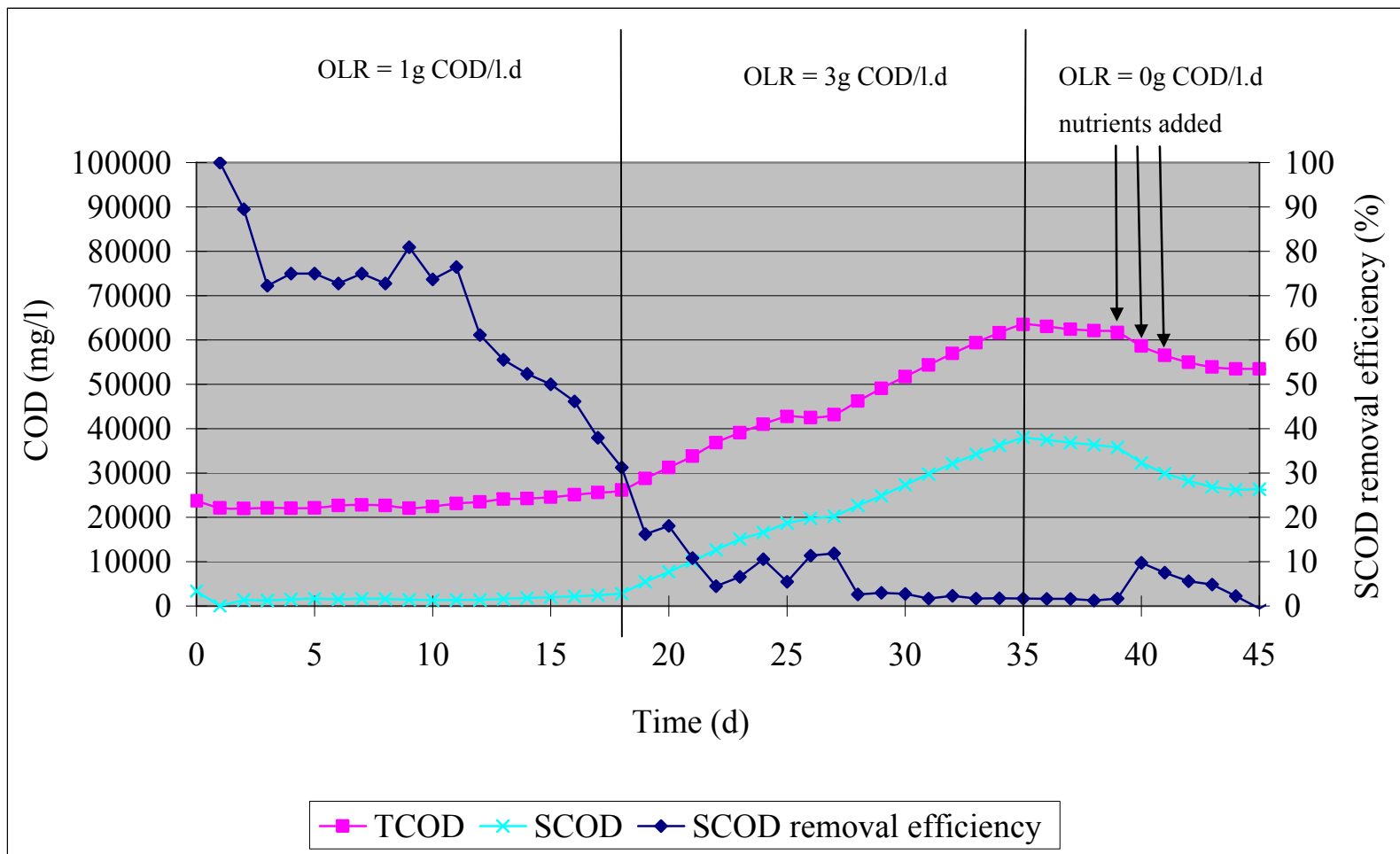


Figure 4.2 TCOD, SCOD and SCOD removal efficiency in the reactor of the semi-continuous experiment (day 0 to day 45).

methanogenic reactor. They suggested that it was necessary to add some micro and macro nutrients to the cheese whey feed solution. They used ammonium bicarbonate and dipotassium hydrogen phosphate to adjust the COD/N/P ratio to 250:5:1. The concentrations of the micro and macro nutrients added to the cheese whey feed solution are given in Table 4.1 (for COD concentration of 20,000 mg/l).

Table 4.1 Nutrient solution composition used by Yilmazer and Yenigun

Macronutrient	Concentration	Micronutrient	Concentration
K ₂ HPO ₄	448 mg/l	FeCl ₃ .6H ₂ O	4.830 mg/l
NH ₄ HCO ₃	2256 mg/l	NiSO ₄ .6H ₂ O	0.444 mg/l
		CoCl ₂ .6H ₂ O	0.402 mg/l
		(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	0.036 mg/l

Yilmazer and Yenigun (1999)

In this study, the C:N:P ratio of whey powder was 100:0.1:0.3, indicating the deficiency of nitrogen and phosphorus in the substrate. This may explain why the SCOD removal efficiency decreased without nutrient supplement and increased after addition of nutrients to the reactor. The high SCOD removal efficiency during first few days of the reaction may be supported by the nutrients and trace element in the digester sludge.

4.1.2. Effect of trace element addition on COD removal

Figure 4.2 shows that the SCOD and TCOD decreased sharply from day 39 to day 42 due to the addition of sufficient nutrients. However, the SCOD and TCOD began to decrease slowly from day 43 to day 130, as shown in Figure 4.3. This was suspected to be caused by insufficient trace element. In order to investigate the effect of trace element addition on SCOD removal efficiency, 45 ml trace element solution was added at day 131 (One litre trace element solution contained: FeCl₃.6H₂O 1.5g, H₃BO₃ 0.15g, CuSO₄.5H₂O 0.03g, KI 0.03g, MnCl₂.4H₂O 0.12g, Na₂MoO₂.2H₂O 0.06 g, ZnSO₄.7H₂O 0.12g, and CoCl₂.6H₂O 0.15g), 27 mg ZnSO₄.7H₂O (= 6.2 mg Zn) was added at day 151, and 76 mg FeCl₃.6H₂O (=15.8 mg Fe) was added at day 156, as shown in Table 3.1.

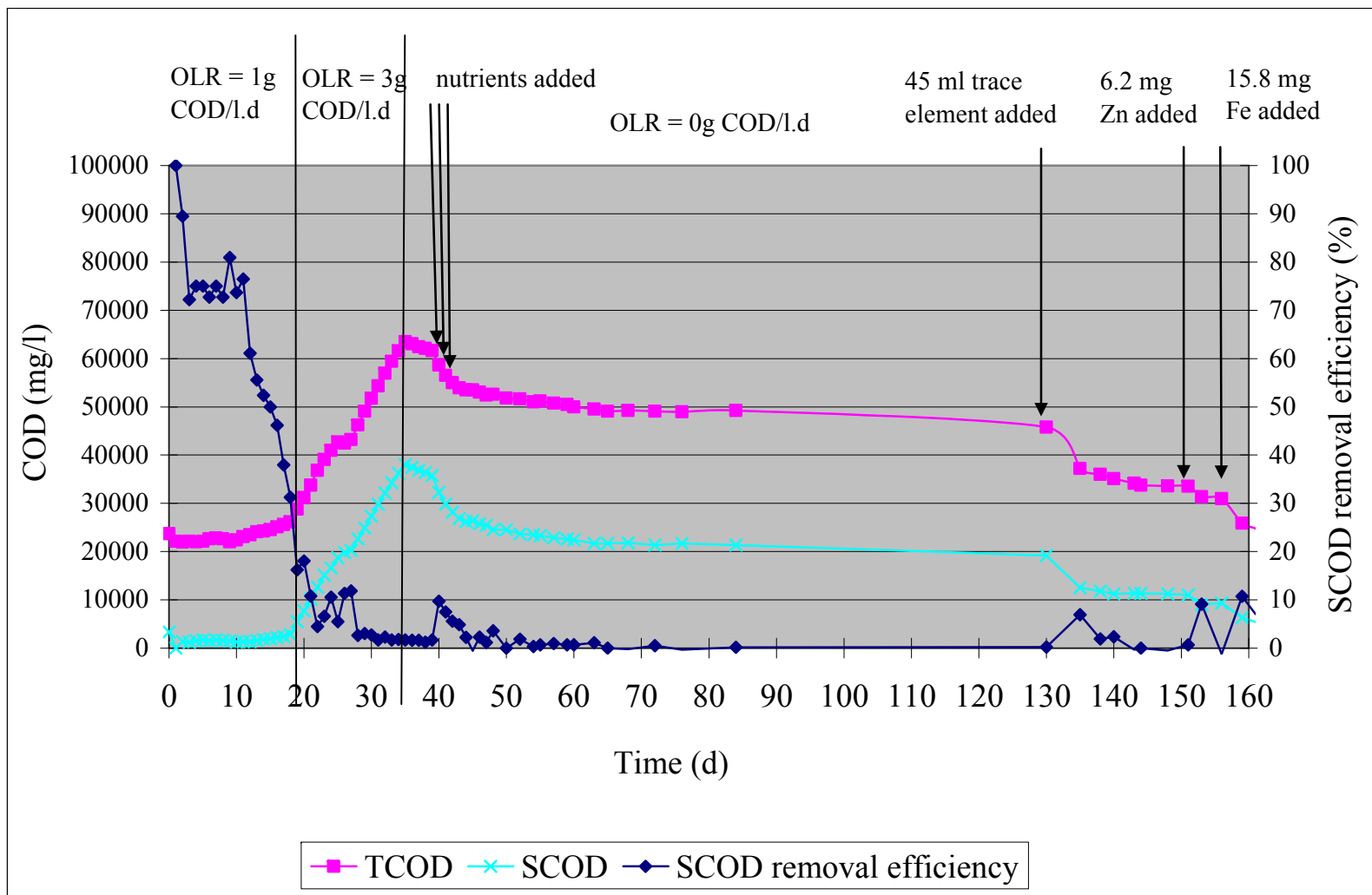


Figure 4.3 TCOD, SCOD and SCOD removal efficiency in the reactor of the semi-continuous experiment (day 0 to day 160).

As can be seen from Figure 4.3, the TCOD and SCOD decreased significantly after trace element solution or metals were added. The SCOD removal efficiency increased to 8.7% after adding 45 ml trace element solution at day 131. Furthermore, the SCOD removal efficiency increased to 9.1% and 16.1% after adding 27mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ at day 151 and 76 mg $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ at day 156, respectively. This indicated that supplement of trace element including Fe^{3+} and Zn^{2+} was also necessary for biological growth.

Trace metals play an important role to stimulate methanogenic activity. Gonzales-Gil et al. (1999) pointed out that metal deficiencies can limit the performance of anaerobic digestion. Metal supplement may substantially stimulate the performance of anaerobic digestion. The most important metal ions in many reactions of anaerobic digestion were Ni, Co and Fe. The recommended requirements for iron, cobalt, nickel and zinc are 0.02, 0.004, 0.003 and 0.02 mg/g acetate produced, respectively. Selenium, molybdenum, manganese, aluminium, and boron have been recommended as additional components in media (Azbar et al., 2000).

Handajani (2004) reported that Fe was an essential metal for improving methanogenesis. Ferric ions addition or the addition of a mix of minerals improved acetate degradation and methane production rates more than two-folds because minerals addition had a positive effect on the yield coefficients. Ferric ions increased the yield coefficient of acetate by 50% and improved the methane and acetate conversion rate by a factor of 1.5. A combination of Ni, Co, Mn and Mg also increased the yield coefficient by 25%. This was in agreement with the current study which showed that the supplement of a mix of minerals, Zn^{2+} and Fe^{3+} improved the SCOD removal efficiency by 8.5%, 8.4% and 16.1%, respectively.

Many other researchers who investigated anaerobic treatment of whey also suggested the supplementation of nutrient and trace metals. Marshal and Timbers (1982) reported that a 500 litre pilot-scale fixed-film reactor receiving full strength whey suffered from inadequate macronutrient and micronutrient deficiency. The substrate used for the study of Switzenbaum and Danskin (1982) consisted of sweet whey powder and ammonium

phosphate as a supplemental nutrient source. Yan et al. (1989) investigated anaerobic digestion of cheese whey using UASB. The composition of nutrients and metals in 14 litres feed solution was: 6.6 g K_2HPO_4 , 1 g NH_4Cl , 0.04 g Fe^{3+} , and 0.01 g Mg^{2+} .

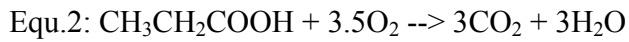
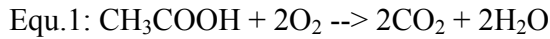
Erguder et al. (2001) compared the supplementation of nutrient and trace metals on gas production of serum bottles by anaerobic digestion of cheese whey. The composition of the basal medium (BM, which contained all the necessary micro and macro nutrients required for an optimum anaerobic microbial growth) used in their experiments was as follows (concentrations of the constituents are given in parentheses as mg/l): NH_4Cl (1200), $MgSO_4 \cdot 7H_2O$ (400), KCl (400), $Na_2S \cdot 9H_2O$ (300), $CaCl_2 \cdot 2H_2O$ (50), $(NH_4)_2HPO_4$ (80), $FeCl_2 \cdot 4H_2O$ (40), $CoCl_2 \cdot 6H_2O$ (10), KI (10), $MnCl_2 \cdot 4H_2O$ (0.5), $CuCl_2 \cdot 2H_2O$ (0.5), $ZnCl_2$ (0.5), $AlCl_3 \cdot 6H_2O$ (0.5), $NaMoO_4 \cdot 2H_2O$ (0.5), H_3BO_3 (0.5), $NiCl_2 \cdot 6H_2O$ (0.5), $NaWO_4 \cdot 2H_2O$ (0.5), Na_2SeO_3 (0.5), cysteine (10), and $NaHCO_3$ (6000). They reported that the serum bottles with BM resulted in higher rates and amounts of gas production compared to that without BM. For the influent COD concentration of 22 g/l, the cultures with and without BM produced 598 and 125 ml of gas, respectively. This indicated the significance of nutrient and trace metal supplementation in the anaerobic treatment of cheese whey. This was in agreement with current study which showed that nutrient and trace metal supplementation was necessary for anaerobic digestion of whey.

4.1.3. The effect of nutrients and heavy metals on Volatile Fatty Acids (VFAs)

In anaerobic digestion, the soluble compounds are first hydrolysed by anaerobic bacteria to simple monomers such as sugar, amino acids and fatty acids. Then, these products are used by bacteria that perform acidogenesis to volatile fatty acids (VFAs) such as acetate, propionate, butyrate and valerate. At last, VFAs are utilized by acetogenic and methanogenic bacteria to produce methane.

In this experiment, acetate and propionate were the main products of VFAs, with very low concentration of butyrate and valerate (0-10 mg/l). The concentrations of acetate and

propionate in the reactor are shown in Figure 4.4. From the oxidation equation (Equation 1 and 2) of acetate and propionate, the COD value of each acid can be estimated.



According to equation 1 and 2, 1 mol/l acetate (= 60 g/l) demands 2 mol/l oxygen (= 64 g/l) and 1 mol/l propionate (= 74 g/l) demands 3.5 mol/l oxygen (= 112 g/l). That means that 1 g/l acetate exerts 1.07 g COD/l and that 1 g/l propionate exerts 1.51 g COD/l. Using these constants, the COD of VFAs can be calculated, as shown in Figure 4.5 (the COD of butyrate and valerate were ignored due to the low concentration). Moreover, the SCOD and the SCOD of other non-VFAs organic compounds in the reactor, which was the difference between SCOD and COD of VFAs (= SCOD – COD of VFAs), are shown in Figure 4.5.

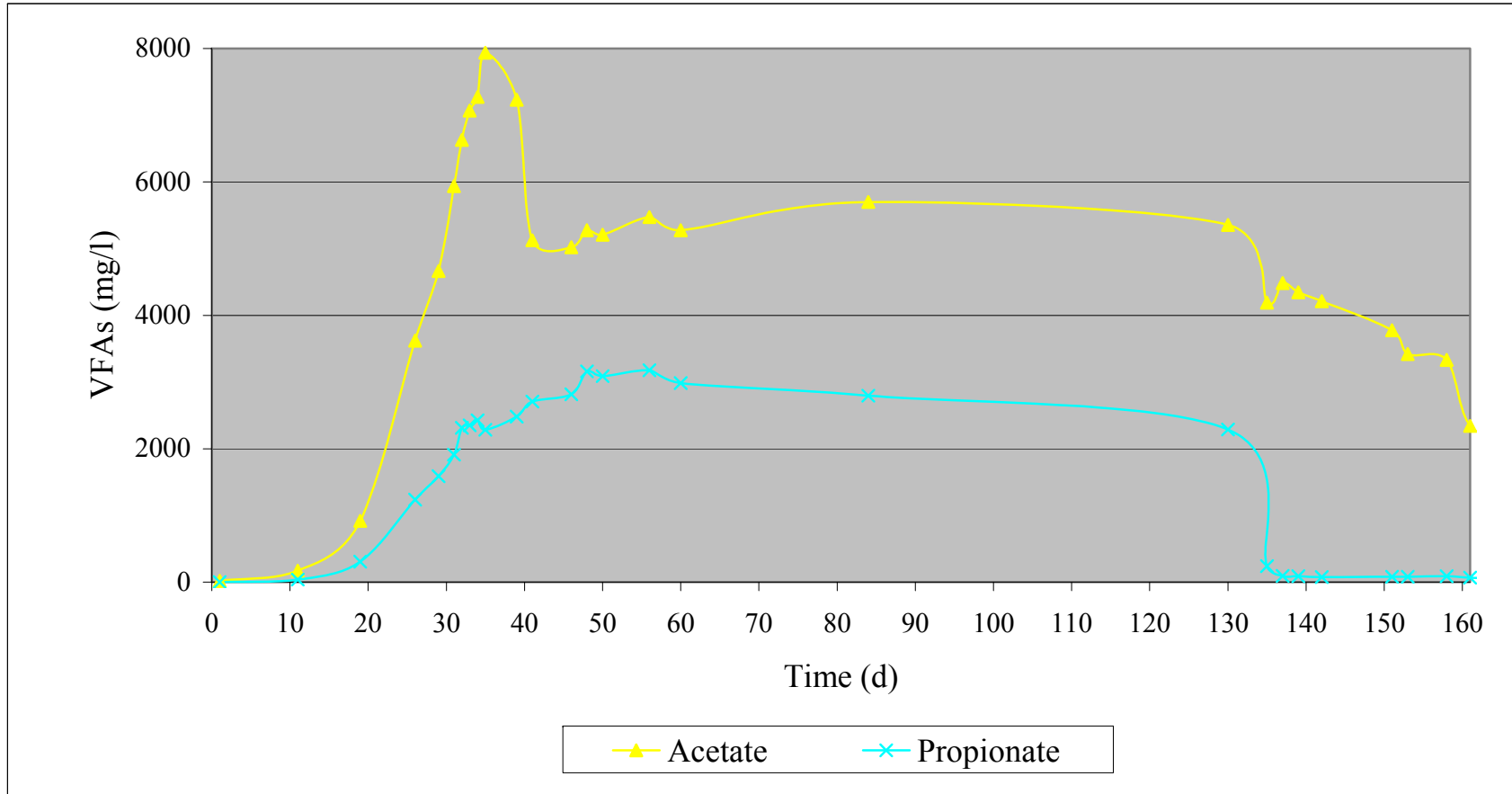


Figure 4.4 VFA concentrations in the reactor of the semi-continuous experiment.

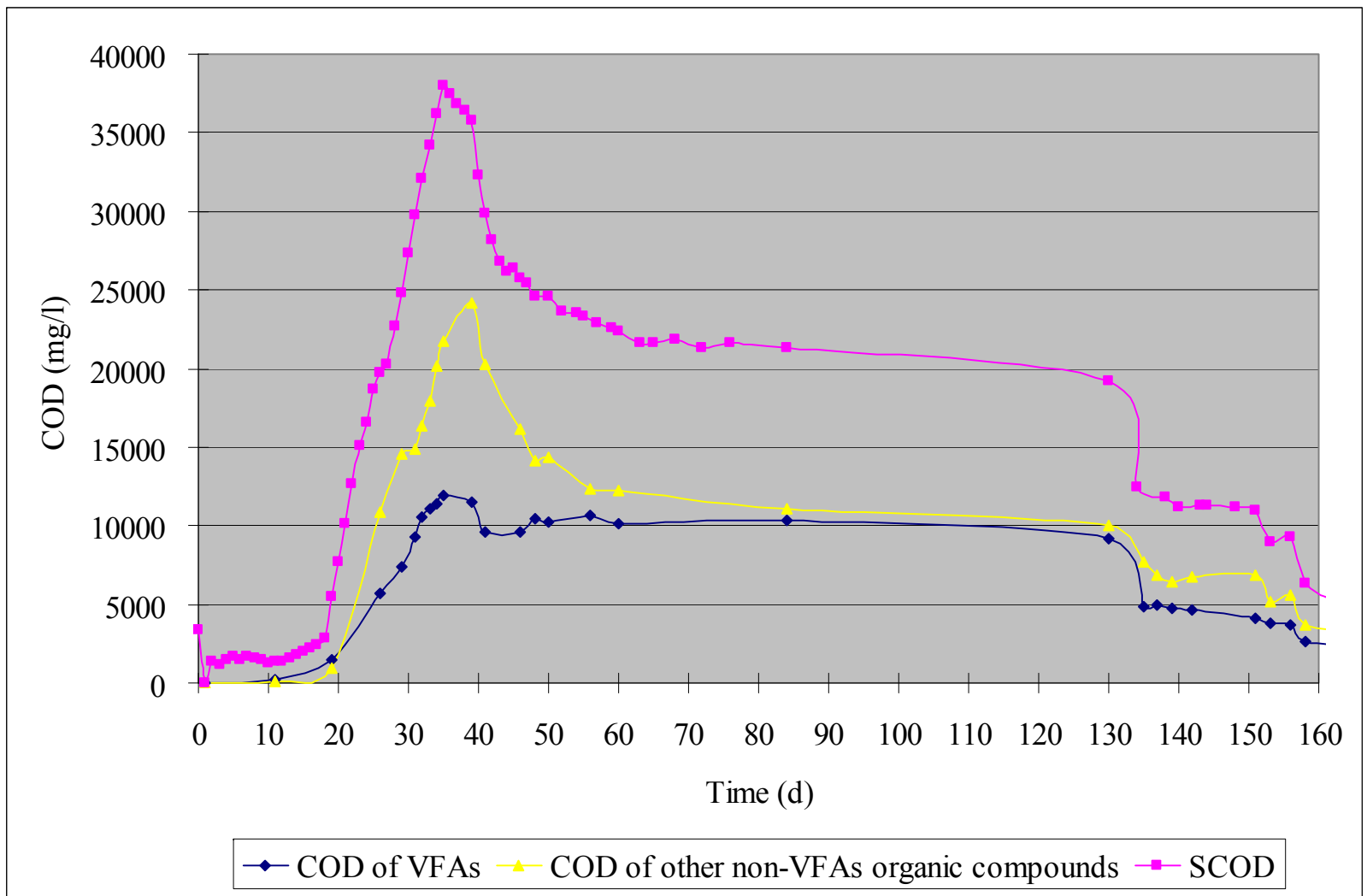


Figure 4.5 Difference between SCOD and COD of VFAs

As can be seen from Figure 4.5, the difference between SCOD and COD of VFAs was quite low during the first 11 days of digestion, and increased a little from day 11 to day 19. From day 19 to day 39, the difference between SCOD and COD of VFAs increased quickly as the OLR changed from 1 to 3 g/l.d. As can be seen from Figure 4.4, from day 26 to day 34 when was 3 g/l.d, acetic acid increased 3.9 g /l and propionic acid increased 1.5 g /l (0.9 gCOD/l.d VFAs were accumulated). However, from day 26 to day 34, SCOD increased 17 g /l (2.1 g/l.d) and TCOD increased 19 g /l (2.4 g/l.d). This indicates that about 0.9 g/l.d SCOD was removed. If all the whey added (3 g/l.d) was converted to VFAs and 0.9 g/l.d was removed, the VFA accumulation should be 2.1 g/l.d. However, only 0.9 gCOD/l.d VFAs were accumulated, which means 1.2 gCOD/l.d whey was not converted to VFAs.

Figure 4.5 shows that VFAs contributed only about half of the SCOD. In other words, only part of the whey powder fed was converted to VFAs, which means not only the acetogenesis and methanogenesis, but also the hydrolysis and acidogenesis, were inhibited without adequate nutrients and minerals addition.

Figure 4.4 shows that from day 35 to day 39, acetic acid decreased 2 g /l and propionic acid increased 0.2 g /l (1.6 gCOD/l.d VFAs were removed). As can be seen from Figure 4.3 and Figure 4.4, from day 35 to day 39, SCOD decreased 2.4 g /l (0.6 g/l.d) and TCOD decreased 1.8 g /l (0.5 g/l.d). From day 39 to day 42 when nutrients were added, SCOD and TCOD decreased quickly. 7.6 g /l (2.5 g/l.d) SCOD and 6.7 g /l (2.2 g/l.d) TCOD were removed while only 2.9 g/l acetic acid and 0.2 g/l propionic acid were degraded (1.1 gCOD/l.d VFAs were removed). From day 43 to day 55, SCOD decreased by 7.1 g /l (0.6 g/l.d) and TCOD decreased by 6.4 g /l (0.5 g/l.d) while VFAs began to increase, which indicated that there were more VFAs accumulated. The accumulation of VFAs indicated that the speed of acetogenesis and methanogenesis were slower than the speed of acidogenesis when nutrients and minerals were limited.

As can be seen from Figure 4.5, there were significant decreases of SCOD, VFAs, as well as the difference between SCOD and COD of VFAs after nutrients and minerals were

supplemented. As discussed before, the sudden decrease of SCOD showed that the addition of nutrients and minerals stimulated the performance of anaerobic digestion system. However, the decreases of VFAs and the difference between SCOD and COD of VFAs after nutrients and minerals addition indicated the speed of acidogenesis were slower than acetogenesis and methanogenesis. This could be explained by that methane producing bacteria were more sensitive to nutrients and minerals addition than acid producing bacteria.

4.2. Co-digestion of primary sludge and whey

The results of the previous semi-continuous whey digestion study showed that nutrients, trace elements as well as metals were necessary to maintain the anaerobic whey digestion system. The main objectives of the co-digestion of primary sludge and whey study were as follows:

- To investigate the feasibility and the performance of co-digestion of primary sludge and whey without addition of nutrients, trace elements and metals.
- To investigate the factors affecting the performance of the co-digestion reactors.
- To investigate the OLR of co-digestion of primary sludge and whey.

During the investigation of anaerobic co-digestion of primary sludge and whey process, the performance of anaerobic digestion of primary sludge alone without pH control at HRT of 20 days was monitored as a control. The OLR of the reactor was 3.3 ± 0.1 g COD/l.d from day 1 to 7 and was 4.0 ± 0.1 g COD/l.d from day 8 due to the COD variation of the primary sludge. The biogas production was 1.3 ± 0.1 l/d from day 1 to 7 and was 1.7 ± 0.1 l/d from day 8. The TCOD removal efficiency was $51 \pm 3\%$ from day 1 to 7 and was $57 \pm 3\%$, from day 8.

4.2.1. The effect of COD loading ratio of primary sludge to whey on the performance of the reactors

The purpose of this experiment was to investigate the feasibility of co-digestion of primary sludge and whey as well as the effect of the COD loading ratio of primary sludge to whey on the reactor performance.

Three anaerobic continuous stirred reactors (namely, reactor A, reactor B and reactor C) with HRT of 20 days were set up. Reactor A was fed with 100 ml primary sludge as a control, while reactor B and reactor C were fed with different volume of primary sludge and whey solution (66 ± 2 g COD/l), with total volume of 100 ml. The volume of primary sludge (PS) and whey solution fed in the three reactors are shown in Table 3.2. The COD

loading ratio of primary sludge to whey and the OLR of each reactor are also shown in Table 3.2. Due to the COD variation of the primary sludge (from day 1 to 7, 66 ± 2 g COD/l; from day 8, 80 ± 2 g COD/l), the OLR of the three reactors were various from day 8.

4.2.1.1. The effect of COD loading ratio of primary sludge to whey on biogas production and COD removal efficiency

From day 1 to 7, reactor A, B and C were operated with similar OLR but various COD loading ratios of primary sludge to whey (as shown in Table 4.2). The performances of the three reactors were monitored by measuring the pH, TCOD, SCOD, bicarbonate alkalinity, VFA and lactate concentrations of the effluent. The performance comparison of Reactor A, B and C from day 1 to 7 is shown in Table 4.2. The TCOD concentration of effluent and TCOD removal efficiency of reactor A, B and C are shown in Figure 4.6. The Biogas production of reactor A, B and C are shown in Figure 4.7.

Table 4.2 The performance comparison of Reactor A, B and C from day 1 to 7

Reactor	A	B	C
pH control	No	No	No
pH	6.9 ± 0.1	6.9 ± 0.1	6.9 ± 0.1
HRT (d)	20	20	20
Feed of primary sludge (ml)	100	70	30
Feed of whey solution (ml)	0	30	70
TCOD concentration of influent (g COD/l)	66 ± 2	66 ± 2	66 ± 2
OLR (g COD/l.d)	3.3 ± 0.1	3.3 ± 0.1	3.3 ± 0.1
COD loading ratio (PS : whey)	100:0	70:30	30:70
TCOD concentration of effluent (g COD/l)	32 ± 2	30 ± 2	28 ± 2
TCOD removal efficiency (%)	51 ± 3	54 ± 3	57 ± 3
Biogas production (l/d)	1.3 ± 0.1	1.5 ± 0.1	1.8 ± 0.1

Table 4.2, Figure 4.6 and Figure 4.7 show that with same HRT and OLR, the biogas production and the TCOD removal efficiency of reactor C were higher than that of

reactor B, and the biogas production and TCOD removal efficiency of reactor B were higher than that of reactor A. As can be seen in Table 4.2, from day 1 to 7, the three reactors were fed at different COD loading ratio of primary sludge to whey. The COD loading ratio of primary sludge to whey of reactor C was 30:70, reactor B was 70:30, and reactor A was 100:0. Therefore, when operated at the same OLR and HRT, digestion of the mixture of primary sludge and whey with higher whey content achieved higher biogas production and higher TCOD removal efficiency. This was similar to the result of the study of Lo et al. (1988) who investigated the anaerobic digestion of a mixture of cheese whey and dairy manure. They reported that biogas production from the mixture was lower than biogas production from cheese whey but higher than biogas production from screened dairy manure.

The result may have been due to the different organic content and biodegradability between whey and primary sludge. As can be seen from Figure 4.6, after feeding with different substrates at similar OLR, the TCOD concentration of the effluent of reactor A was higher than that of reactor B, and the TCOD concentration of the effluent of reactor B was higher than that of reactor C. This indicates that the TCOD reduction of reactor A was lower than that of reactor B, and the TCOD reduction of reactor B was lower than that of reactor C because the biodegradability of whey was much higher than that of primary sludge. This can explain why the TCOD removal efficiency of reactor A was lower than that of reactor B, and the TCOD removal efficiency of reactor B was lower than that of reactor C.

The biogas production potential of a particular organic waste is dependent on the biodegradable fraction of the volatile solids. The expected biogas production is higher when the biodegradable fraction is larger (Lo et al., 1988). This was in agreement with the experimental data. The biogas production potential was higher for the mixture with higher whey content as the biodegradability of whey was much higher than that of primary sludge. This can explain why the biogas production of reactor A was less than reactor B, and that of reactor B was less than reactor C.

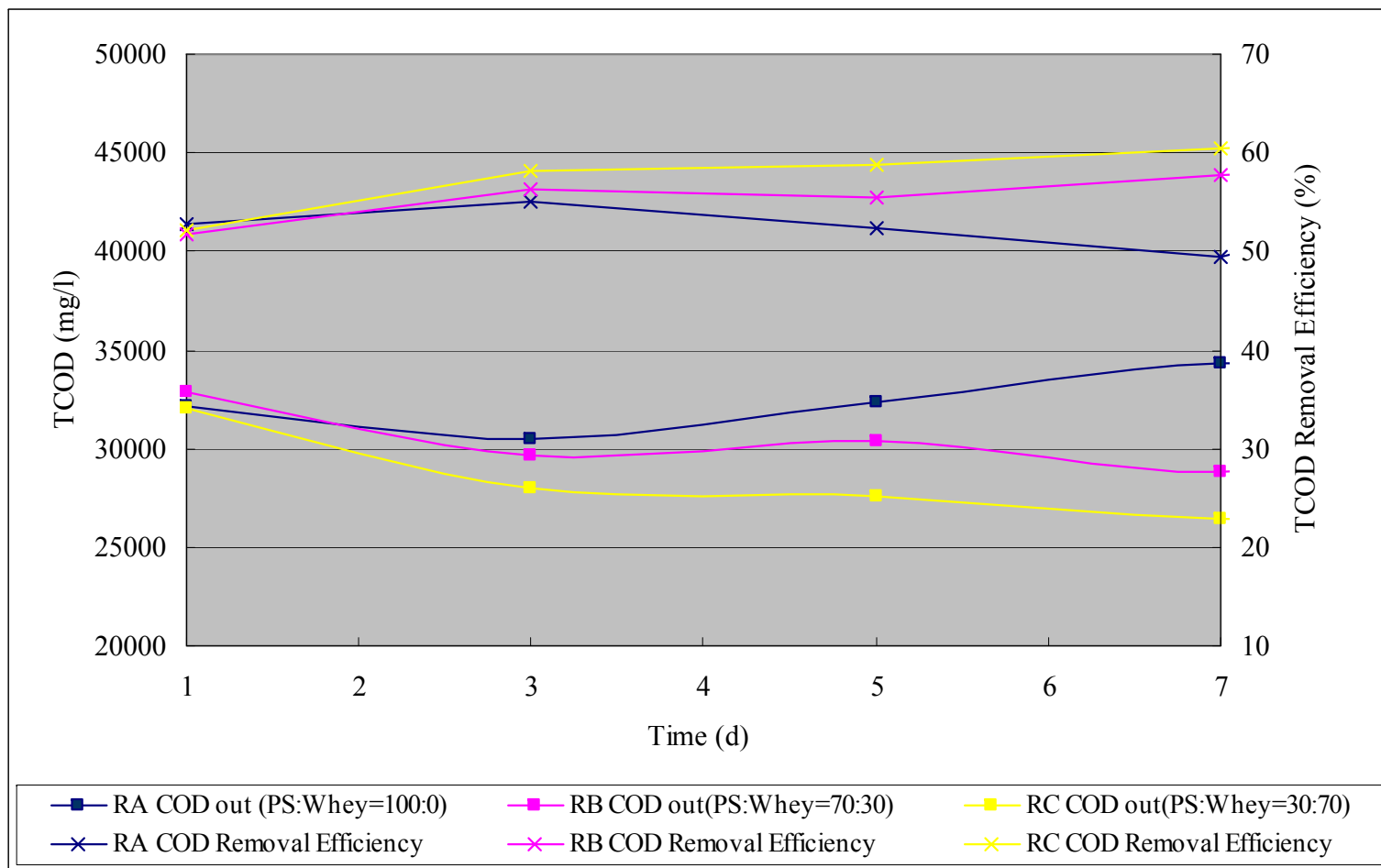


Figure 4.6 TCOD concentration of effluent and TCOD removal efficiency of reactor A, B and C.

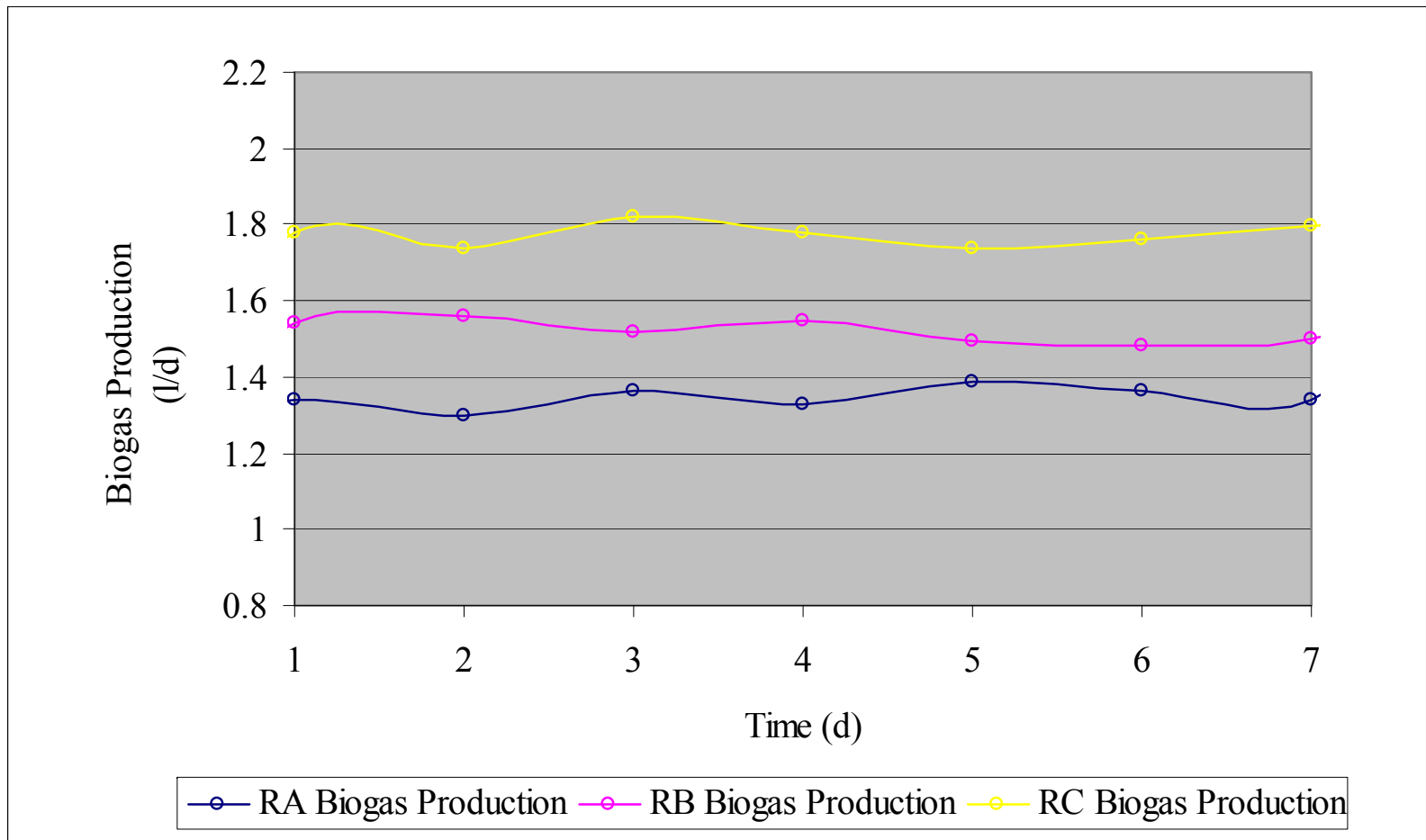


Figure 4.7 Biogas production of reactor A, B and C.

4.2.1.2. The effect of COD loading ratio of primary sludge to whey on the stability of the reactors

From day 8 to 30, due to the COD concentration of the primary sludge increased from 66 ± 2 g COD/l to 80 ± 2 g COD/l, the OLR and the COD loading ratio of primary sludge to whey of the three reactors changed slightly as shown in Table 4.3.

Table 4.3 Feed comparison of reactor A, reactor B and reactor C (from day 8 to 30)

Time (d)	Reactor	HRT (d)	Feed		OLR (g COD/l.d)	COD loading ratio (PS : whey)	pH control
			PS (ml)	whey solution (ml)			
Day 8-30	A	20	100	0	4.0 ± 0.1	100:0	No
	B	20	70	30	3.8 ± 0.1	69:31	No
	C	20	30	70	3.5 ± 0.1	33:67	No

The pH and bicarbonate alkalinity of reactor A, B and C are shown in Figure 4.8 and Figure 4.9, respectively.

As can be seen from Figure 4.8 and 4.9, during 30 days reaction, the pH of reactor A ranged from 6.8 to 7.0 without pH control, while the bicarbonate alkalinity increased from 1325 to 1925 mg CaCO₃/l. The pH of reactor B remained between 6.7 and 6.9 without pH control, and the bicarbonate alkalinity remained between 1375 and 1550 mg CaCO₃/l. However, the pH of reactor C decreased from 6.9 to 6.5, and the bicarbonate alkalinity decreased from 1425 to 950 mg CaCO₃/l. Therefore, the performance of reactor A and reactor B were more stable than the performance of reactor C. This might due to the different COD loading ratio of primary sludge to whey.

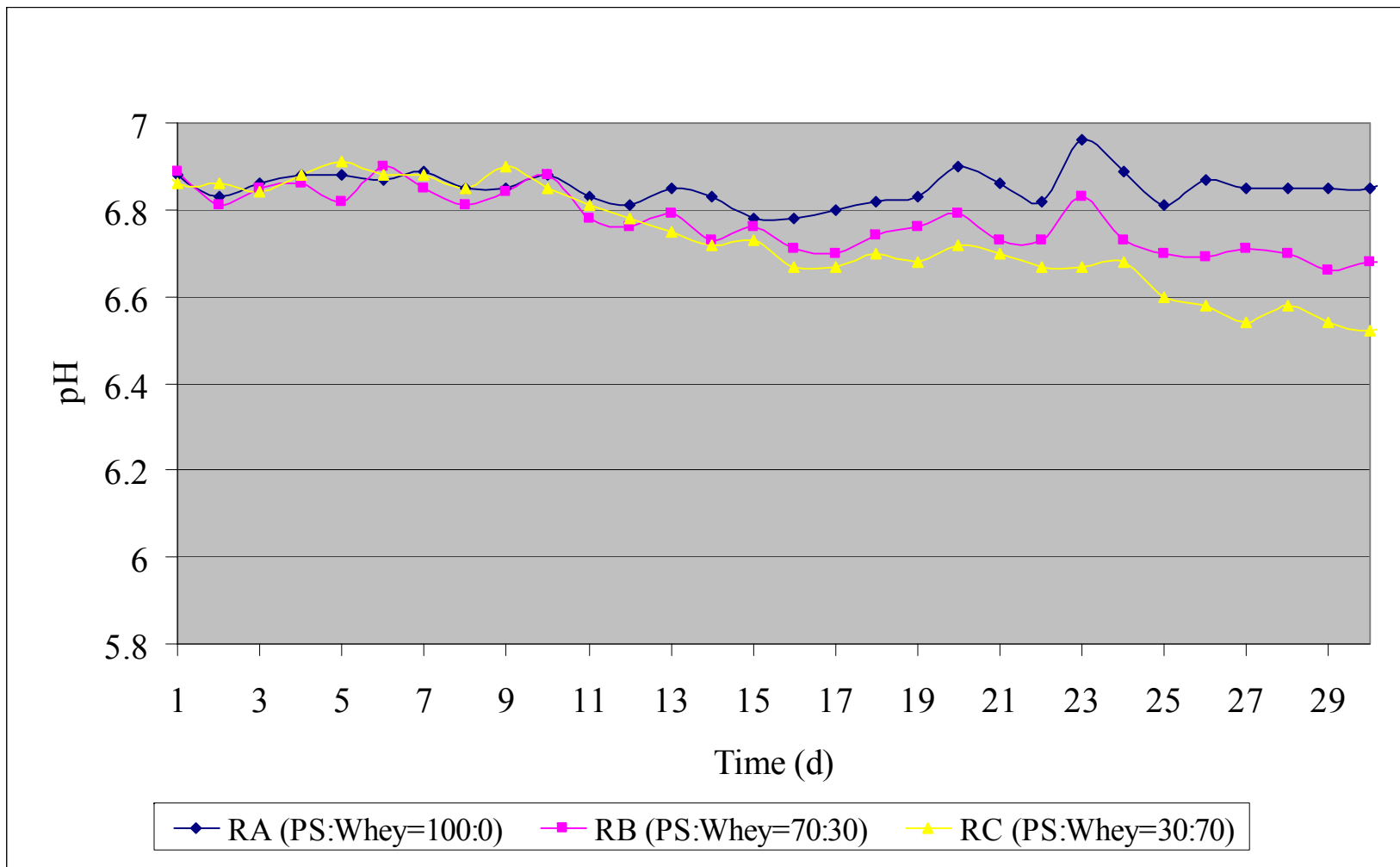


Figure 4.8 The pH of reactor A, B and C.

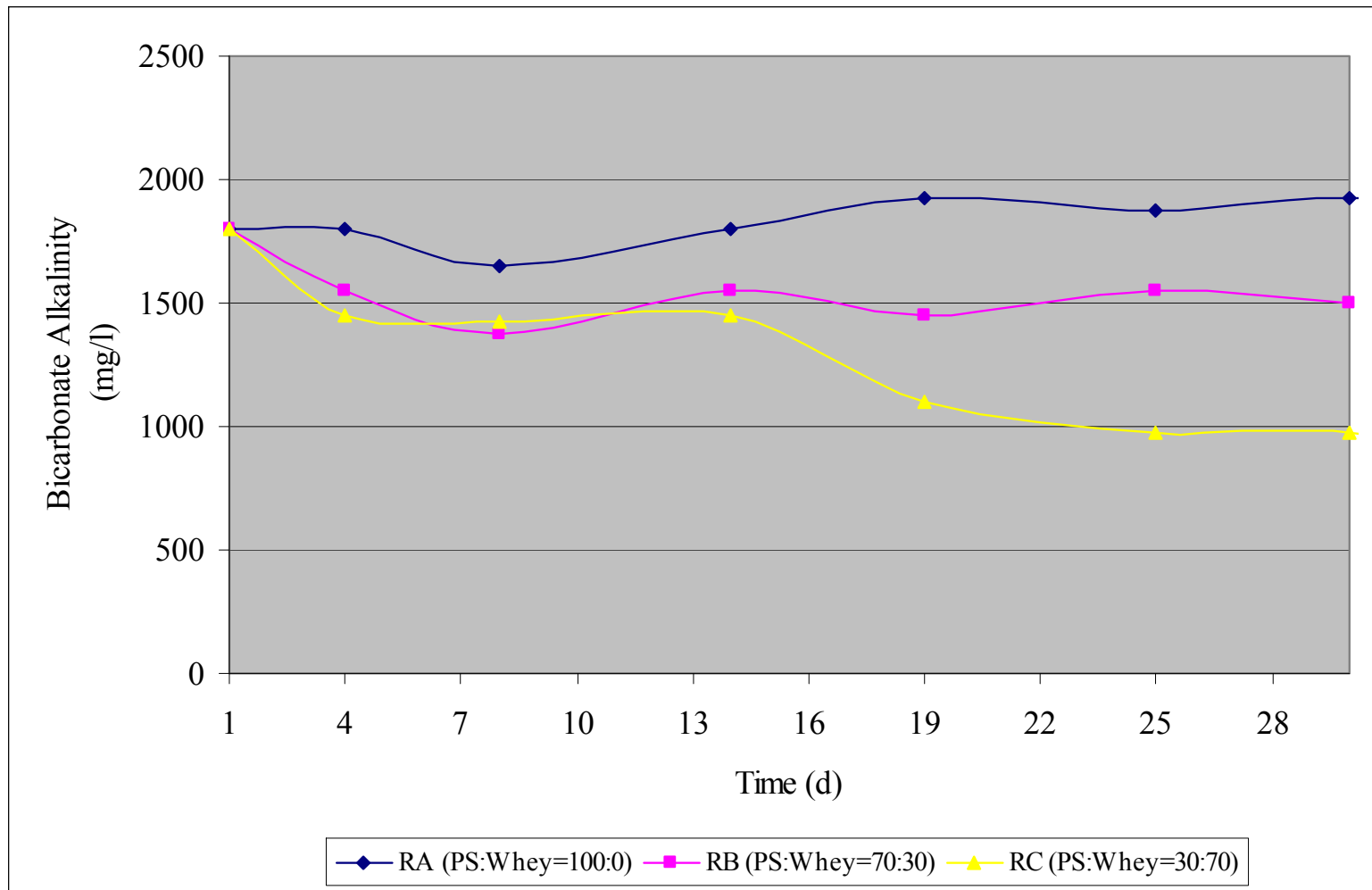


Figure 4.9 The bicarbonate alkalinity of reactor A, B and C.

According to the results of previous study of semi-continuous anaerobic whey digestion, nutrient and trace metal supplementation as well as pH control were necessary for anaerobic digestion of whey. However, the performance of reactor B showed that with proper COD loading ratio of primary sludge to whey (70:30), it was feasible to digest a mixture of primary sludge and whey without nutrient and trace metal supplement and pH control. The primary sludge may served as nutrient supplement and buffering reagent. Nevertheless, when the COD loading ratio of primary sludge to whey was too low (30:70), reactor C appeared unstable, due to the deficiency of nutrient and trace metal or buffering reagent supplement.

4.2.2. The effect of pH on co-digestion of primary sludge and whey

4.2.2.1. Comparison of the effect of pH on the performance of reactor C and reactor C' (similar OLR to reactor C with pH control)

Since reactor C appeared unstable and its pH dropped from 6.9 to 5.9 at day 63, reactor C was changed to reactor C' in that the pH was controlled between 6.8 and 7.0 by adding sodium bicarbonate (NaHCO₃) from day 64 to investigate the effect of pH on the performance of co-digestion of primary sludge and whey. The feed comparison of reactor C and reactor C' is shown in Table 4.4.

Table 4.4 Feed comparison of reactor C and reactor C'

Reactor	HRT (d)	Feed		COD loading ratio (PS : whey)	OLR (g COD/l.d)	pH control
		PS (ml)	Whey solution (ml)			
C	20	30	70	33:67	3.5 ± 0.1	No
C'	20	30	70	33:67	3.5 ± 0.1	Yes (6.9 ± 0.1)

The biogas production and pH of reactor C and C' are shown in Figure 4.10, which shows that without pH control, the pH of reactor C kept decreasing due to the high ratio of whey in the substrate. The biogas production of reactor C started decreasing as pH

dropped to 6.3. While for reactor C' with pH control, the biogas production was recovered.

The VFA concentration and pH of reactor C and C' are shown in Figure 4.11. As can be seen from Figure 4.11, the total VFA concentrations increased with the decreasing of the pH. More specifically, without alkalinity addition, the acetate and propionate began to accumulate when the pH dropped to 6.3, followed by iso-butyrate and valerate accumulating at pH of 6.2. However, after alkalinity addition, the system was recovered due to the fact that the VFA concentrations decreased and biogas production increased. Iso-butyrate and valerate concentrations began to decrease when pH increased to 6.6, and then acetate and propionate concentrations started to drop when pH increased to 6.9. No butyrate and only a small amount of iso-valerate was found in the reactor. Although the major component of whey is lactose, only a very small amount lactic acid was found in the co-digestion reactors. Based on the report of Yang et al. (1987), it is possible that lactate is not observed to be present because it is immediately converted to acetic acid and other compounds.

With alkalinity addition to maintain the pH between 6.8 and 7.0, the performance of reactor C' was better than the performance of reactor C without alkalinity addition. This is in agreement with Ghaly (1996) who suggested that the biogas production proceeds quite well as long as the pH are maintained between 6.6 and 7.6 with an optimum range between 7.0 and 7.2.

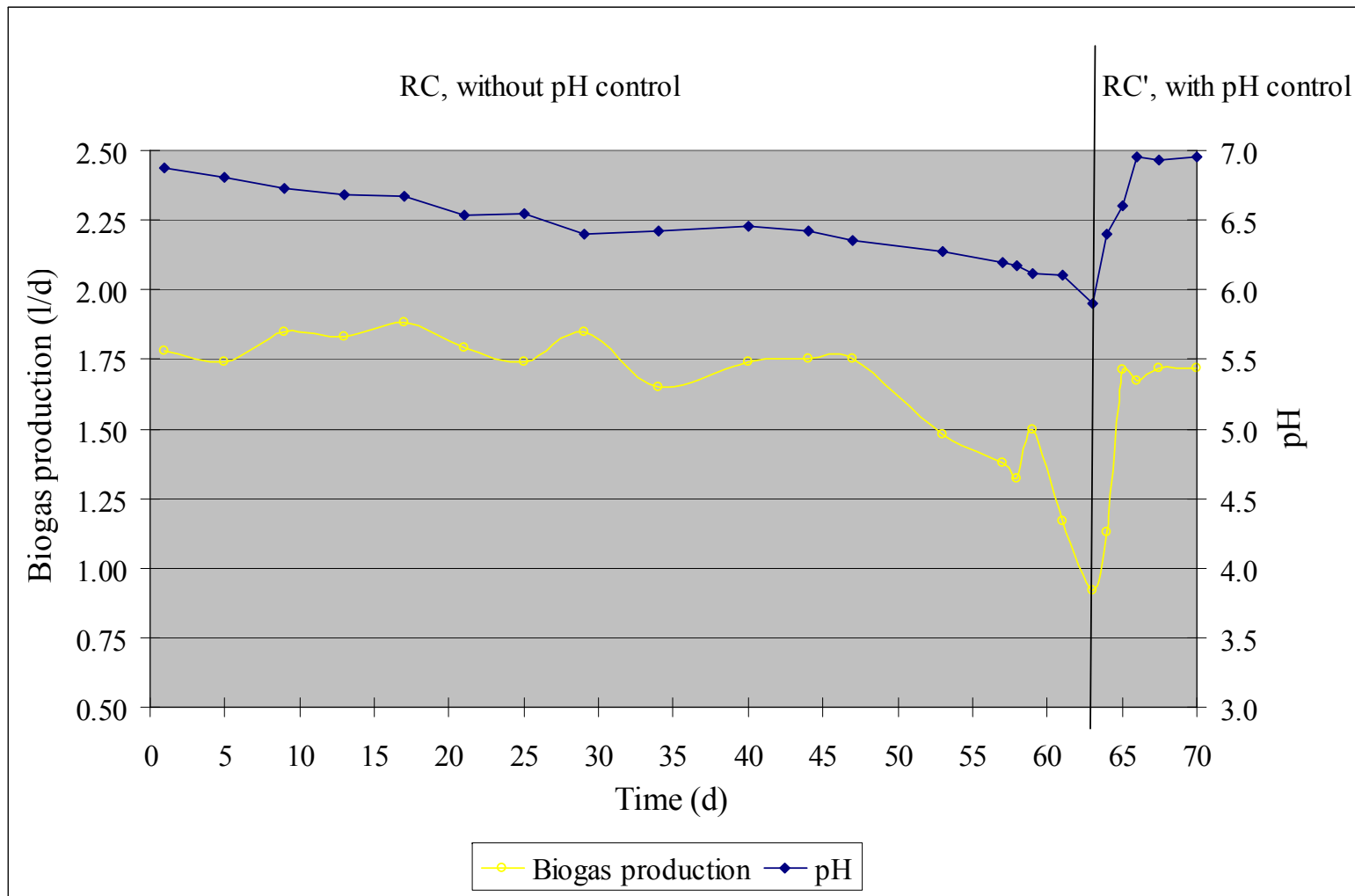


Figure 4.10 Biogas production and pH of reactor C (without pH control) and reactor C' (with pH control).

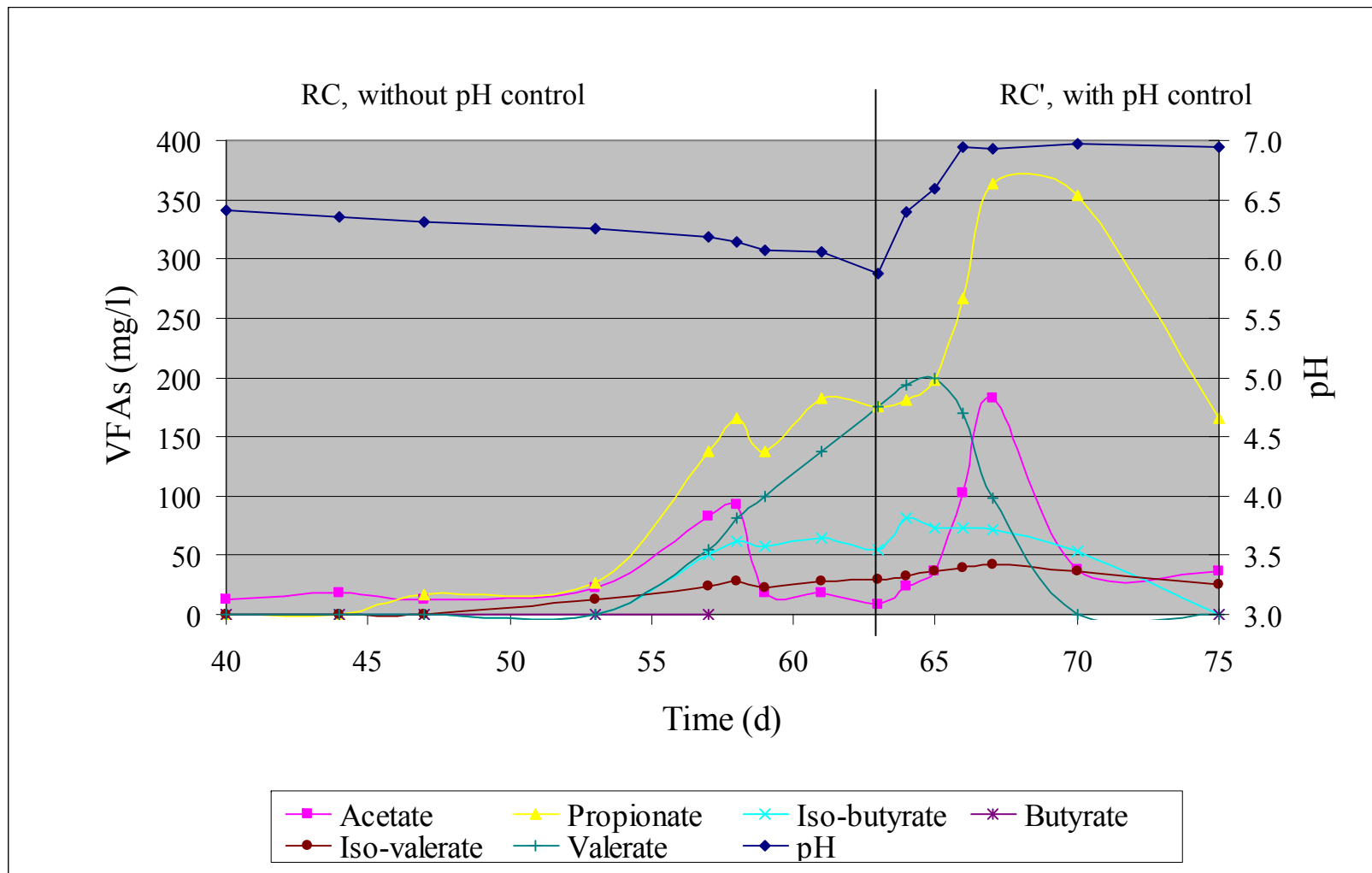


Figure 4.11 VFA concentrations and pH of reactor C (without pH control) and reactor C' (with pH control).

4.2.2.2. Comparison of the effect of pH on the performance of reactor D and reactor D' (similar OLR to reactor D with pH control)

The purpose of the experiment was to redo reactor C and reactor C' at higher OLR to further investigate the effect of pH on the performance of two reactors (reactor D without pH control and reactor D' with pH control).

The HRT of each reactor was 20 days. Reactor D was fed with 43 ml primary sludge and 57 ml whey solution (145 ± 2 g COD/l) per day without pH control. Reactor D' treated similar substrate to reactor D but with pH control ($\text{pH} = 6.9 \pm 0.1$). The OLR and the COD loading ratio of primary sludge to whey of the reactors are also shown in Table 4.5.

Table 4.5 Feed comparison of reactor D and reactor D'

Reactor	HRT (d)	Feed		COD loading ratio (PS : whey)	OLR (g COD/l.d)	pH control
		PS (ml)	whey solution (ml)			
D	20	43	57	30:70	5.8 ± 0.1	No
D'	20	43	57	30:70	5.8 ± 0.1	Yes (6.9 ± 0.1)

The pH, bicarbonate alkalinity concentration, total VFA concentration and biogas production comparisons of reactor D and reactor D' are shown in Figure 4.12 to Figure 4.15. As can be seen from Figure 4.12 to Figure 4.15, during the 27 days of co-digestion, the pH of reactor D decreased from 6.8 to 4.5, while the bicarbonate alkalinity decreased from 1700 to 0 mg CaCO₃/l. The total VFAs in reactor D accumulated from 12 to 3883 mg/l as the decreasing of the bicarbonate alkalinity and pH. Biogas production of reactor D dropped from 3.3 to 0.4 l/d. Therefore, the performance of reactor D was unstable. On the contrary, the performance of reactor D' was very stable due to the pH of reactor D' was controlled between 6.8 and 7.0. The bicarbonate alkalinity of reactor D' remained between 1700 and 2100 mg/l and no VFA accumulated. Biogas production of reactor D' remained between 2.9 and 3.2 l/d.

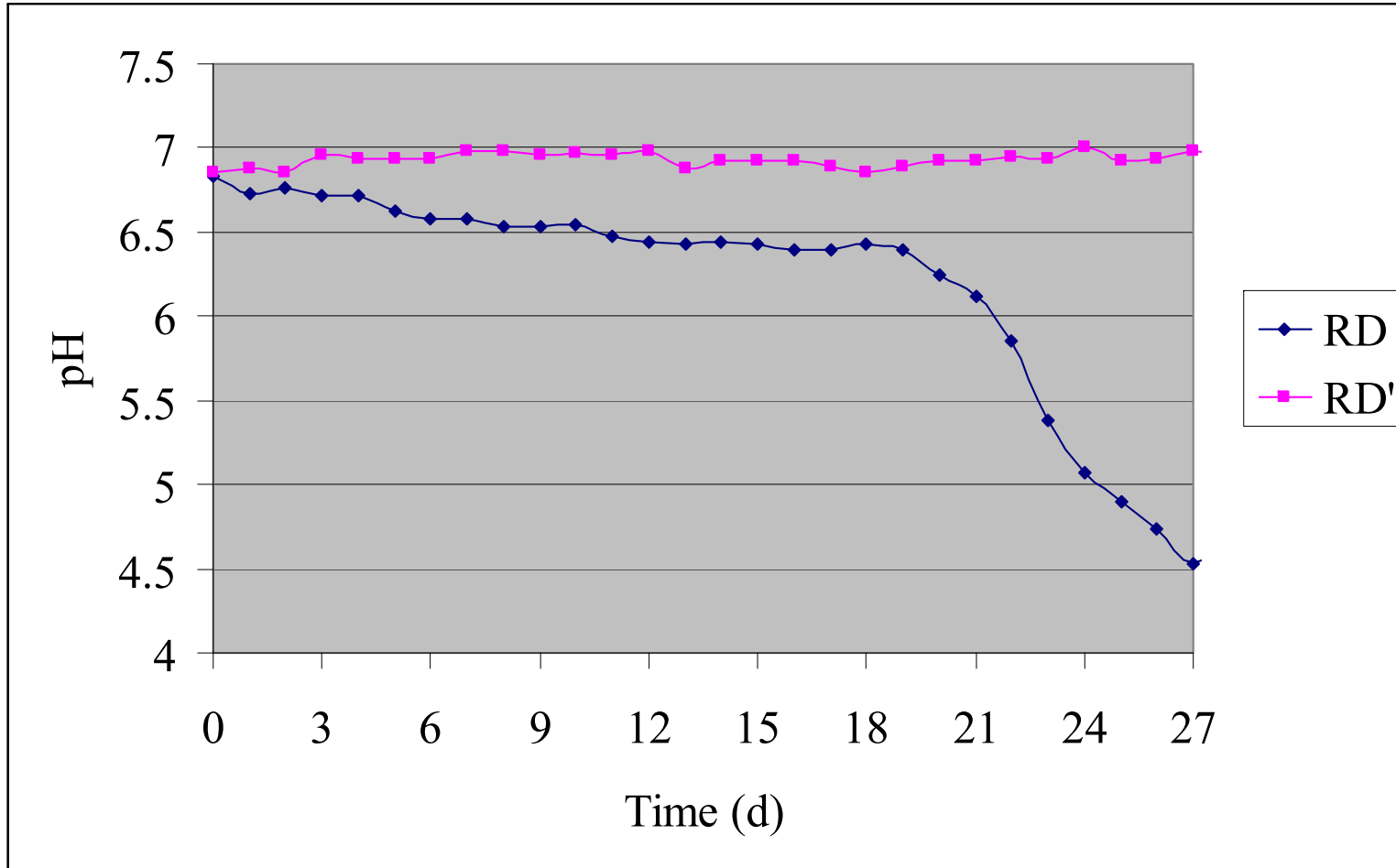


Figure 4.12 pH of reactor D and reactor D'.

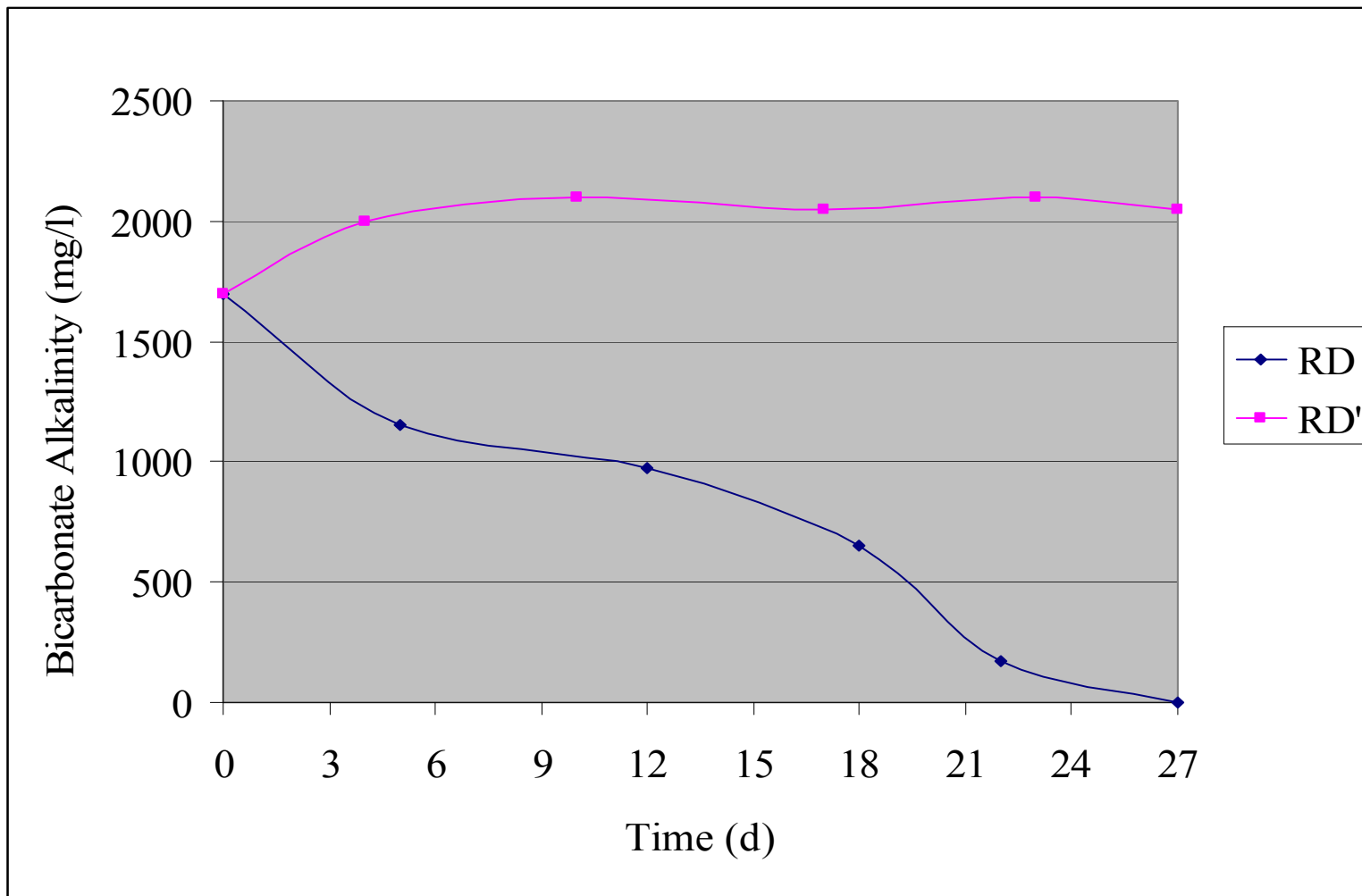


Figure 4.13 Bicarbonate alkalinity concentration of reactor D and reactor D'.

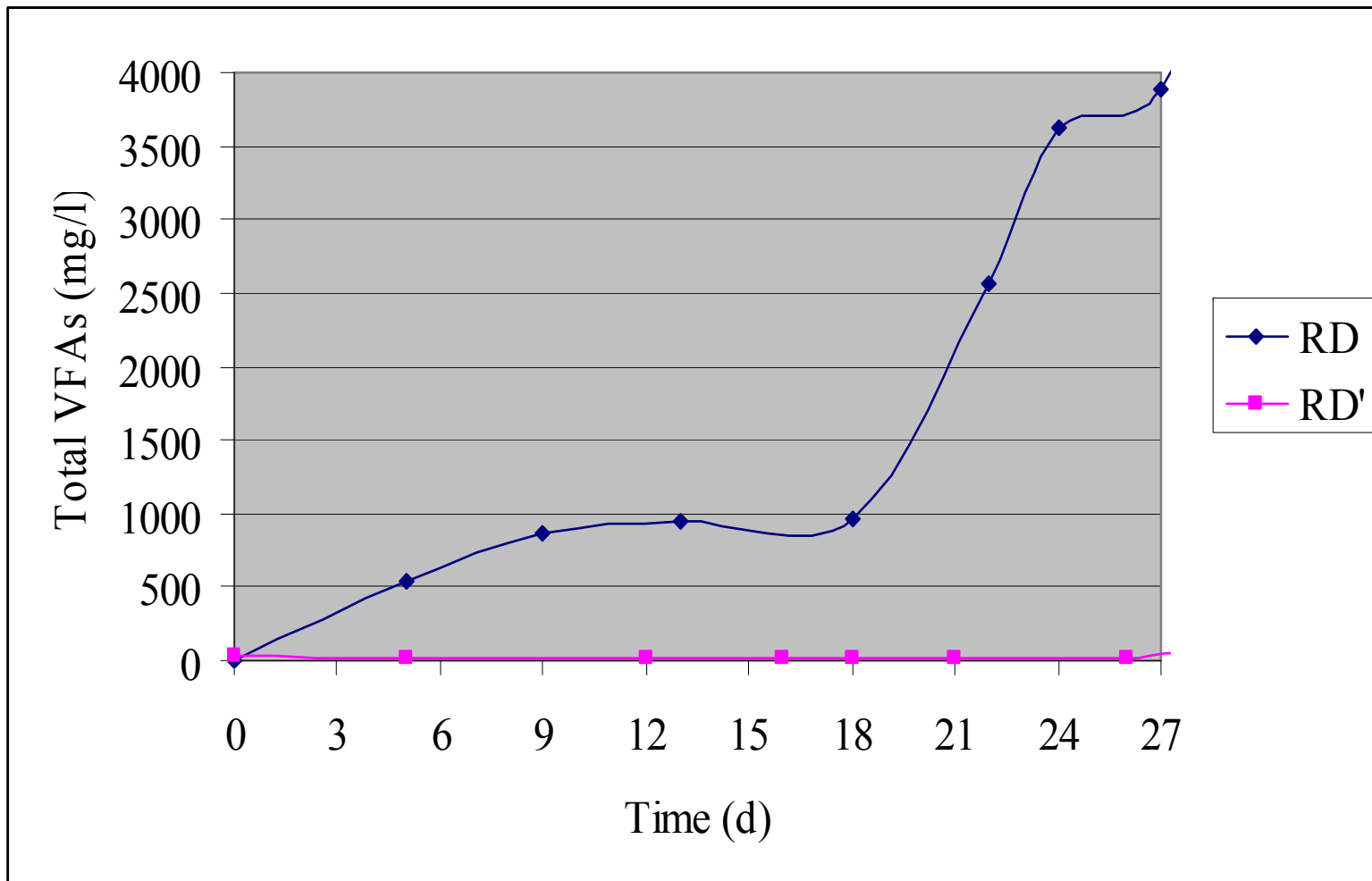


Figure 4.14 The total VFA concentrations of reactor D and reactor D'.

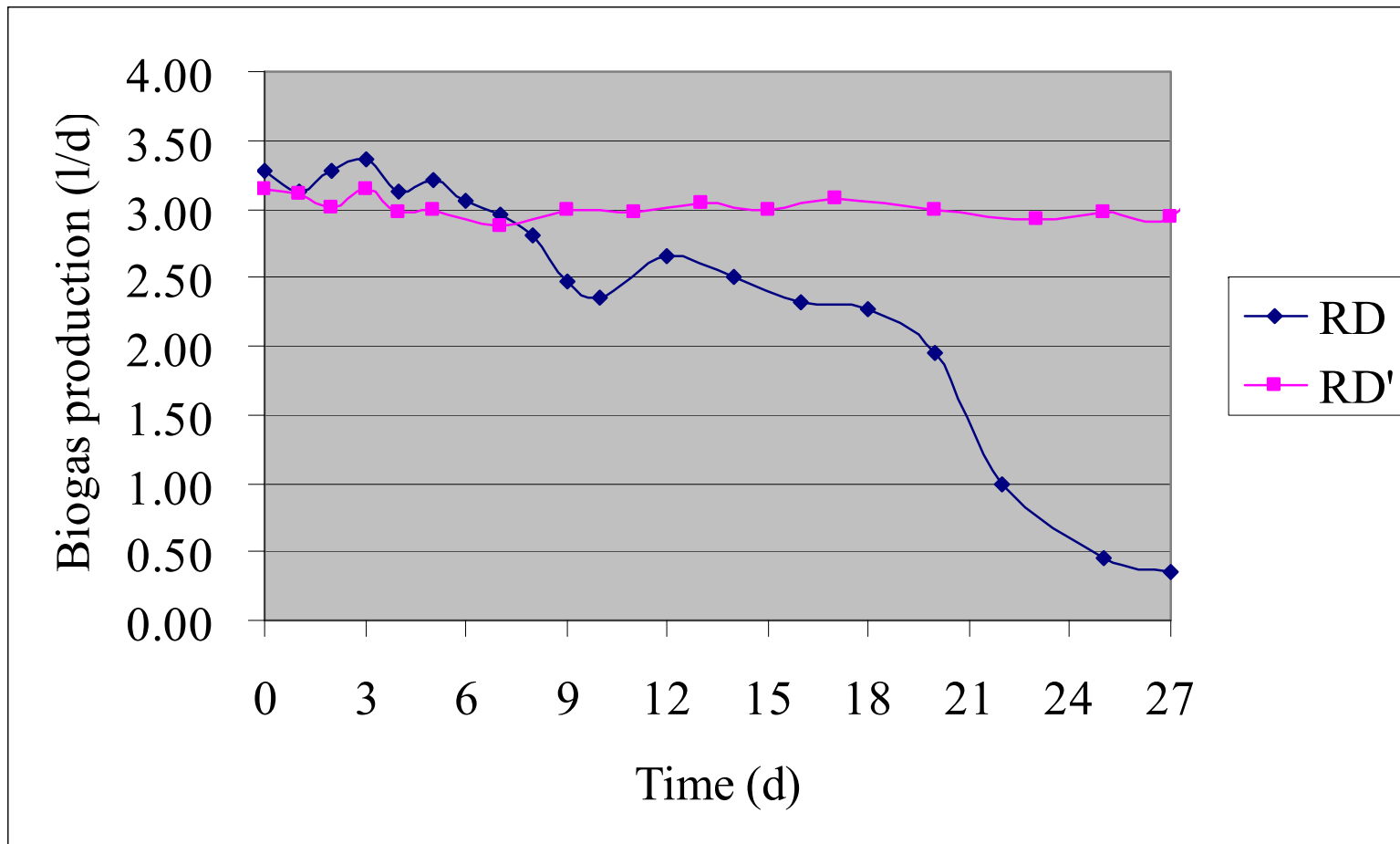


Figure 4.15 The biogas production of reactor D and reactor D'.

Comparing the performance of reactor D and Reactor D', the biogas production of reactor D and Reactor D' were similar when pH values were above 6.8. However, reactor D failed when the pH kept dropping from 6.8 to 4.5. This may be because the anaerobic digestion system is limited to a relatively narrow pH interval from approx. 6.0 to 8.5, a pH value outside this range can lead to imbalance of the system. In most anaerobic digesters, a neutral condition, as indicated by an average pH of 6.8-7.2 is considered normal (Water Pollution Control Federation, 1987). Wildenauer and Winter (1985) reported that in conventional mixed reactors at pH values below 6.6 acute toxicity occurs. This was in agreement with the result of the current study that the performance of reactor D showed unstable from day 19 when the pH dropped to 6.4.

pH is strongly dependent on the buffering capacity, or alkalinity, of the system (Ahring et al., 1995). The pH decrease of reactor D may be caused by the low bicarbonate alkalinity of whey. Whey has very high biodegradability (about 99%) and tends to rapidly acidify to VFAs (Mockaitis et al., 2005). The treatment of whey by anaerobic degradation was constrained by the drop in pH that inhibited further conversion of acids to methane. Norstedt and Thomas (1984) found that without pH control, an anaerobic fixed-bed reactor could not achieve stable operation within 30 days. Supplemental alkalinity is required so as to avoid anaerobic process failure (Mockaitis et al., 2005). Marshal and Timbers (1982) reported that a 500 l pilot-scale fixed-film reactor receiving raw whey needed the addition of NaOH for pH control. This was in agreement with the current study that when loading ratio of primary sludge to whey was about 30:70, pH control was necessary to maintain the system stable.

The VFA concentrations and pH of reactor D are shown in Figure 4.16. As can be seen from Figure 4.16, the pH of reactor D decreased slowly from 6.9 to 6.1 during the first 21 days of digestion, while the pH dropped sharply from 6.1 to 4.5 in only 5 days (day 22 to day 27). This may be because each of the microbial groups involved in anaerobic degradation has a specific pH optimum and can grow in a specific pH range. The methanogens and acetogens have pH optimum at approx. 7, while acidogens have lower pH optimum around 6, and methanogens at pH lower than 6.6 grow very slowly (Ghaly,

1996). When the pH was about 6, the activities of acidogens were stimulated while the activities of methanogens and acetogens were inhibited, which resulted in the accumulation of VFAs.

Figure 4.16 shows that the first accumulated VFA was propionate. Then some amount of the propionate was transferred to iso-butyrate, valerate, as well as a small amount of iso-valerate. The concentrations of acetate, propionate, butyrate, valerate increased quickly when the pH was below 6.3. However, the fact that acetate only began to accumulate from day 19 may indicate that acetogens are more sensitive to pH than methanogens.

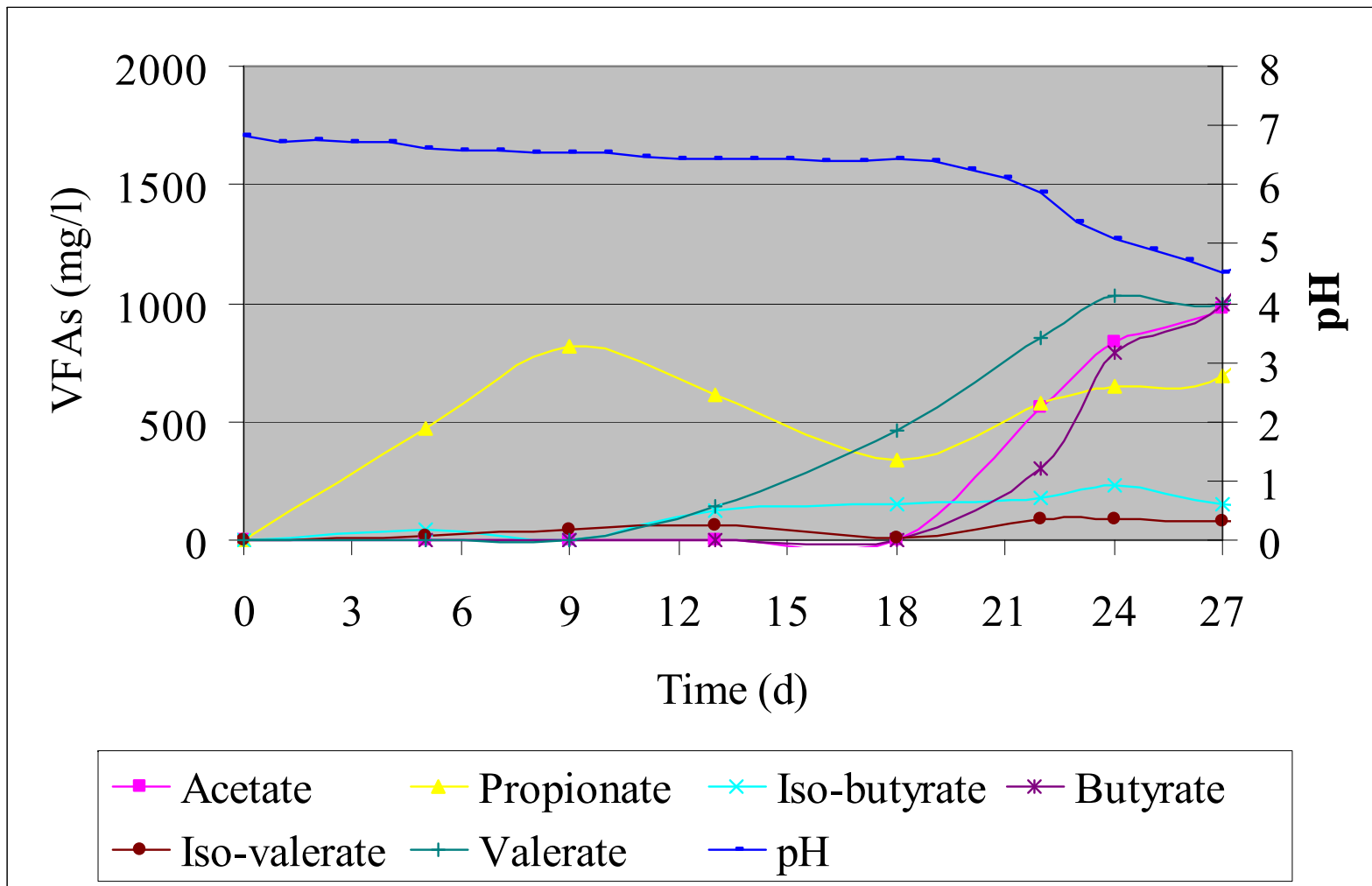


Figure 4.16 The VFA concentrations and pH of reactor D.

4.2.3. Investigation of OLR for co-digestion of primary sludge and whey without pH control

As discussed in 4.2.1.1, when the COD loading ratio of primary sludge to whey was approximately 70: 30, reactor B was stable without pH control due to the primary sludge served as buffer agent. In order to increase OLR for co-digestion of primary sludge and whey without pH control, reactor F with same COD loading ratio of primary sludge to whey as reactor B but higher OLR than reactor B was set up. HRT of reactor F was 20 days. The pH, TCOD, SCOD, bicarbonate alkalinity and VFA concentrations of the effluent were measured.

The performance comparison of reactor A and reactor F is shown in Table 4.6. The biogas production and pH of reactor F are shown in Figure 4.17.

Table 4.6 The performance comparison of reactor A and reactor F

Reactor	A	F
HRT (d)	20	20
pH control	No	No
pH	6.9 ± 0.1	6.7 ± 0.1
Feed of primary sludge (ml)	100	100
Feed of whey powder (g)	0	1.9
TCOD concentration of influent (g COD/l)	80 ± 2	117 ± 2
OLR (g COD/l.d)	4.0 ± 0.1	5.8 ± 0.1
COD loading ratio (PS : whey)	100:0	70:30
TCOD concentration of effluent (g COD/l)	36 ± 2	38 ± 2
TCOD removal efficiency (%)	57 ± 3	68 ± 3
SCOD concentration of effluent (g COD/l)	1.1 ± 0.2	1.6 ± 0.2
Effluent VFA concentration (mg/l)	13 ± 2	18 ± 6
Biogas production (l/d)	1.7 ± 0.1	2.5 ± 0.1

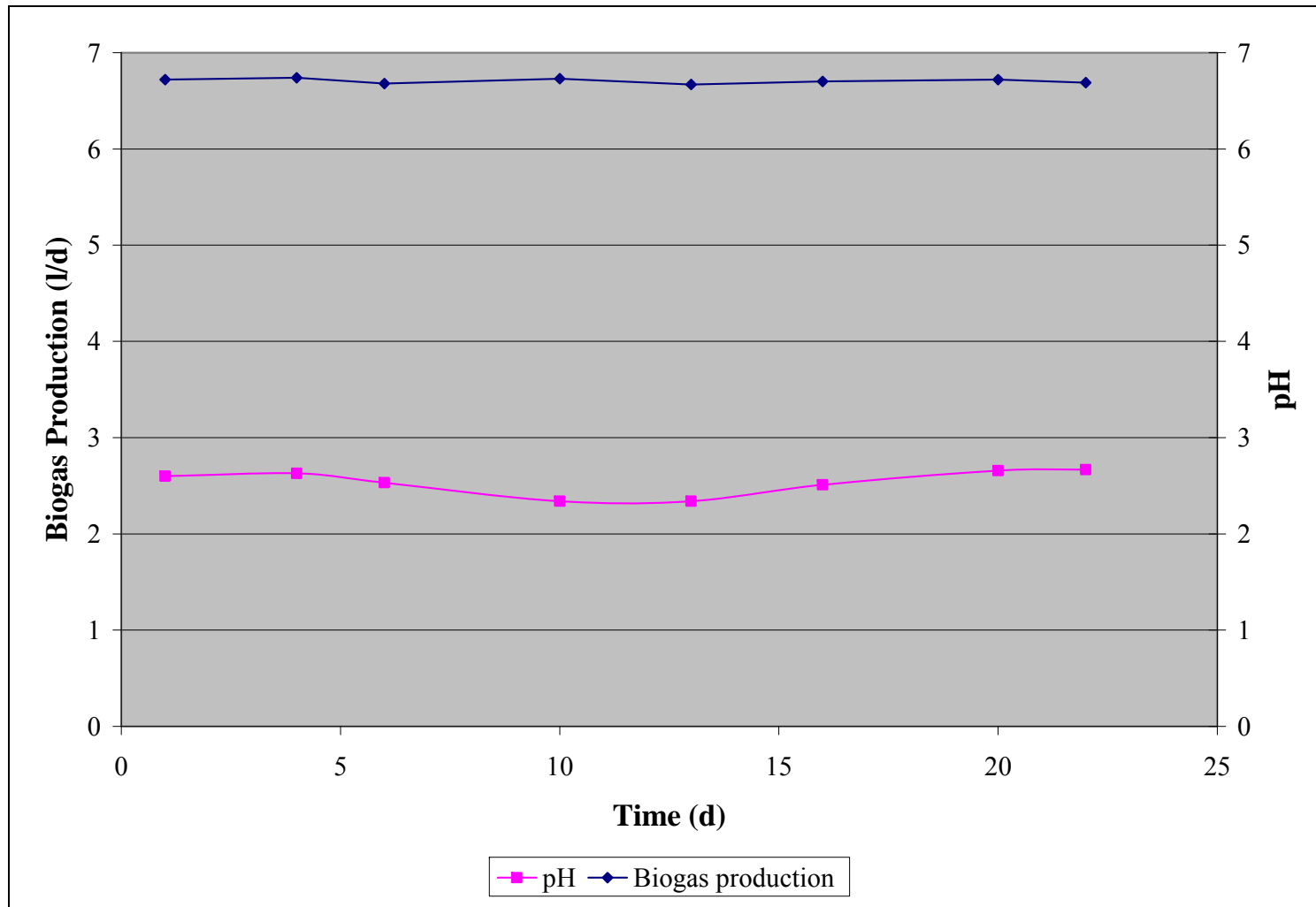


Figure 4.17 The biogas production and pH of reactor F.

As can be seen from Table 4.6 and Figure 4.17, reactor F at OLR of 5.8 ± 0.1 g COD/l.d was quite stable without pH control. Few VFAs accumulated in the reactor, and the pH remained at 6.7 ± 0.1 . However, the effluent TCOD, SCOD and VFA concentrations of reactor F were slightly higher than which of reactor A. This suggested that higher OLR than 5.8 ± 0.1 g COD/l.d would result in over loading and exceeding the assimilative capacity of the methanogenic stage. Therefore, the OLR for co-digestion of primary sludge and whey without pH control could reach 5.8 ± 0.1 g COD/l.d at COD loading ratio of primary sludge to whey of 70:30.

Table 4.6 shows that the OLR of reactor A which only digested 100 ml primary sludge per day was approximately 4.0 ± 0.1 g COD/l.d. The biogas production of reactor A was 1.7 ± 0.2 l/d and the TCOD removal efficiency of reactor A was $57 \pm 3\%$. By feeding additional whey powder without pH control, the OLR could increase to 5.8 ± 0.1 g COD/l.d (reactor F) at COD loading ratio of primary sludge and whey of 70:30. The biogas production increased to 2.5 ± 0.1 l/d and the TCOD removal efficiency increased to $68 \pm 3\%$. Comparing to the performance of the reactor treating same amount of primary sludge alone at same HRT of 20 days, the OLR increased by 1.8 g COD/l.d, the biogas production increased by 0.8 l/d and the TCOD removal efficiency increased by 11 %.

4.2.4. Increasing OLR for co-digestion of primary sludge and whey with pH control

In order to determine what further increase in OLR for co-digestion of primary sludge and whey could be achieved with pH control, other three reactors (namely reactor G, reactor H and reactor I) at various OLR were set up. HRT of the reactors were 20 days. The feed comparison of reactor G, reactor H and reactor I is shown in Table 4.7.

Table 4.7 Feed comparison of reactor G, reactor H and reactor I

Reactor	HRT (d)	Feed		COD loading ratio (PS : whey)	OLR (g COD/l.d)	pH control
		PS (ml)	whey powder(g)			
G	20	100	3.8	53 : 47	7.7 ± 0.1	Yes (6.9±0.1)
H	20	100	4.4	48 : 52	8.1 ± 0.1	Yes (6.9±0.1)
I	20	100	5.5	44 : 56	9.4 ± 0.1	Yes (6.9±0.1)

The performance comparison of reactor A, reactor G, reactor H and reactor I is shown in Table 4.8. The biogas productions of reactor G, reactor H and reactor I are shown in Figure 4.18. As can be seen from Table 4.8 and Figure 4.18, the performances of reactor G and reactor H were quite stable with OLR of 7.6 ± 0.1 and 8.1 ± 0.1 g COD/l.d respectively. However, for reactor I with OLR of 9.4 ± 0.1 , instability was observed which demonstrated by the decrease in gas production, the increases in effluent TCOD and SCOD concentrations, and the accumulation of VFAs in the reactor after 8 days of operation. This indicated that the system could not cope with OLR above 9.4 g COD/l.d. The decrease of the biogas production of reactor I implied that the accumulation of VFAs in the acidogenic stage beyond the assimilative capacity of the methanogenic stage, which was in agreement with what Valentini et al. (1997) suggested that methanogenesis is the rate-limiting step of the overall anaerobic digestion process.

The experimental results indicated that the OLR for co-digestion of primary sludge and whey with pH control could reach to 8.1 ± 0.1 g COD/l.d. The biogas production were 3.5 ± 0.1 l/d, which corresponded to the biogas production rates of 1.8 ± 0.1 litre biogas per litre reactor per day at OLR of 8.1 ± 0.1 g COD/l.d. Lo et al. (1988) studied mesophilic anaerobic digestion of a mixture of cheese whey and dairy manure using an anaerobic rotating biological contact reactor. They reported that approximate 2.8 litre biogas per litre reactor per day was produced at OLR of approximately 7.2 g COD/l.d. The biogas production rate was higher than the biogas production in the current study. This may be because the biodegradability of dairy manure was higher than that of primary sludge.

Table 4.8 Performance comparison of reactor A, reactor G, reactor H and reactor I

Reactor	A	G	H	I
HRT (d)	20	20	20	20
pH control	No	Yes	Yes	Yes
pH	6.9 ± 0.1	6.9 ± 0.1	6.9 ± 0.1	6.9 ± 0.1
Feed of primary sludge (ml)	100	100	100	100
Feed of whey powder (g)	0	3.8	4.4	5.5
TCOD concentration of influent (g COD/l)	80 ± 2	151 ± 1	163 ± 1	188 ± 2
OLR (g COD/l.d)	4.0 ± 0.1	7.6 ± 0.1	8.1 ± 0.1	9.4 ± 0.1
COD loading ratio (PS : whey)	100:0	53:47	49:51	44:56
TCOD concentration of effluent (g COD/l)	36 ± 2	37 ± 2	37 ± 2	Increased from 38 to 55
TCOD removal efficiency (%)	57 ± 3	72 ± 2	77 ± 1	Decreased from 79 to 69
SCOD concentration of effluent (g COD/l)	1.1 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	Increased from 1.6 to 6.2
Total VFA concentration (mg/l)	13 ± 2	14 ± 2	14 ± 2	Increased from 63 to 5959
Biogas production (l/d)	1.7 ± 0.1	3.3 ± 0.1	3.5 ± 0.1	Decreased from 3.8 to 2.1
Stability	Stable	Stable	Stable	Unstable

As can be seen from table 4.8, the OLR of reactor A which only digested 100 ml primary sludge per day was approximately 4.0 ± 0.1 g COD/l.d. The biogas production of reactor A was 1.7 ± 0.2 l/d and the TCOD removal efficiency of reactor A was 57 ± 3%. By feeding additional whey powder with pH control, the OLR could increase to as high as 8.1 ± 0.1 g COD/l.d (reactor H) at COD loading ratio of primary sludge to whey of 49:51. The biogas production increased to 3.5 ± 0.1 l/d and the TCOD removal efficiency increased to 77 ± 1%. Comparing to the performance of the reactor treating same amount of primary sludge alone at same HRT of 20 days, the OLR increased by 4.1 g COD/l.d, the biogas production increased by 1.8 l/d and the TCOD removal efficiency increased by 20%.

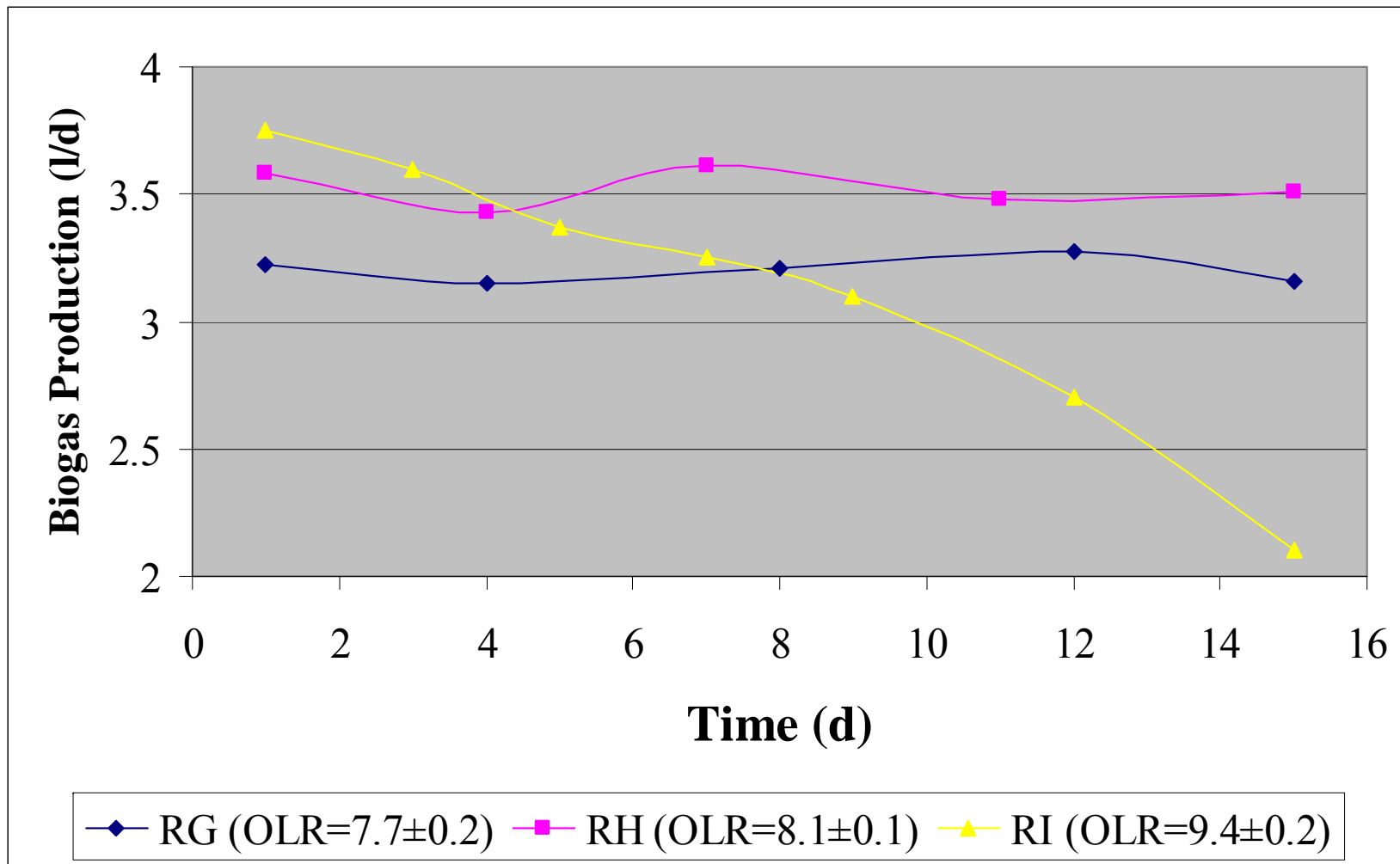


Figure 4.18 Biogas production of reactor G, reactor H and reactor I.

4.2.5. Investigation of the effect of OLR on co-digestion of primary sludge and whey with different HRT

In previous co-digestion of primary sludge and whey experiments (reactor F, G, H and I), the OLR was increased by adding more whey powder, so that the HRT of each reactor remained 20 days. However, in reality whey is presented as liquid. In order to increase OLR using primary sludge and whey solution as substrate to simulate the realistic situation, decreasing HRT was necessary. The purpose of this experiment was to investigate the effect of OLR on co-digestion of primary sludge and whey with different HRT.

Reactor J was set up to treat 100 ml primary sludge and whey solution (66 ± 2 g COD/l) which increased by 2 ml each day gradually from 2 ml to 82 ml during the first 40 days of digestion without pH control. The OLR of Reactor J increased from 3.2 to 6.7 g COD/l.d and the HRT of Reactor J decreased from 20 days to 11 days. The performance of reactor J is shown in Table 4.9.

Table 4.9 The performance of reactor J

Reactor	J
Feed of primary sludge (ml)	100
Feed of whey solution (ml)	Increased from 2 to 82
HRT (d)	Decreased from 20 to 11
OLR (g COD/l.d)	Increased from 3.2 to 6.7
COD loading ratio (PS : whey)	From 98:2 to 61:39
TCOD concentration of effluent (g COD/l)	30 ± 2
TCOD removal efficiency (%)	Increased from 54.7 to 62.2
SCOD concentration of effluent (g COD/l)	1.4 ± 0.3
Effluent VFA concentration (mg/l)	18 ± 6
Biogas production (l/d)	Increased from 1.2 to 2.8

The biogas production of reactor J is shown in Figure 4.19. The effect of OLR on TCOD removal efficiency and biogas production of reactor J is shown in Figure 4.20. The experiment results demonstrated that the biogas production and the TCOD removal efficiency increased with the increase of the OLR (from 3.2 to 6.7 g COD/l.d). This was in agreement with Yan et al. (1988) studying biogas production from cheese whey. They reported that biogas production is dependent on the organic loading rate. The biogas production increased with an increase of the organic loading rate (between 0.7 and 8.3 g COD/l.d).

As can be seen from Figure 4.20, the relationship between the OLR and the biogas production can be expressed by the following equation:

$$y = 0.4499x - 0.2317$$

Where y is biogas production, x is OLR. ($R^2 = 0.9337$)

The experiment results of reactor J indicated that by co-digestion of primary sludge and additional whey solution, the OLR, the biogas production as well as the TCOD removal efficiency increased comparing to digestion of same amount of primary sludge alone. This suggested that the anaerobic sludge digester had the ability to digest additional whey solution and produce more biogas without disturbing the performance of the original reactor. Accordingly, co-digestion of primary sludge and whey solution could be implemented in large scale digestion systems to optimize anaerobic sludge digesters in wastewater treatment plants.

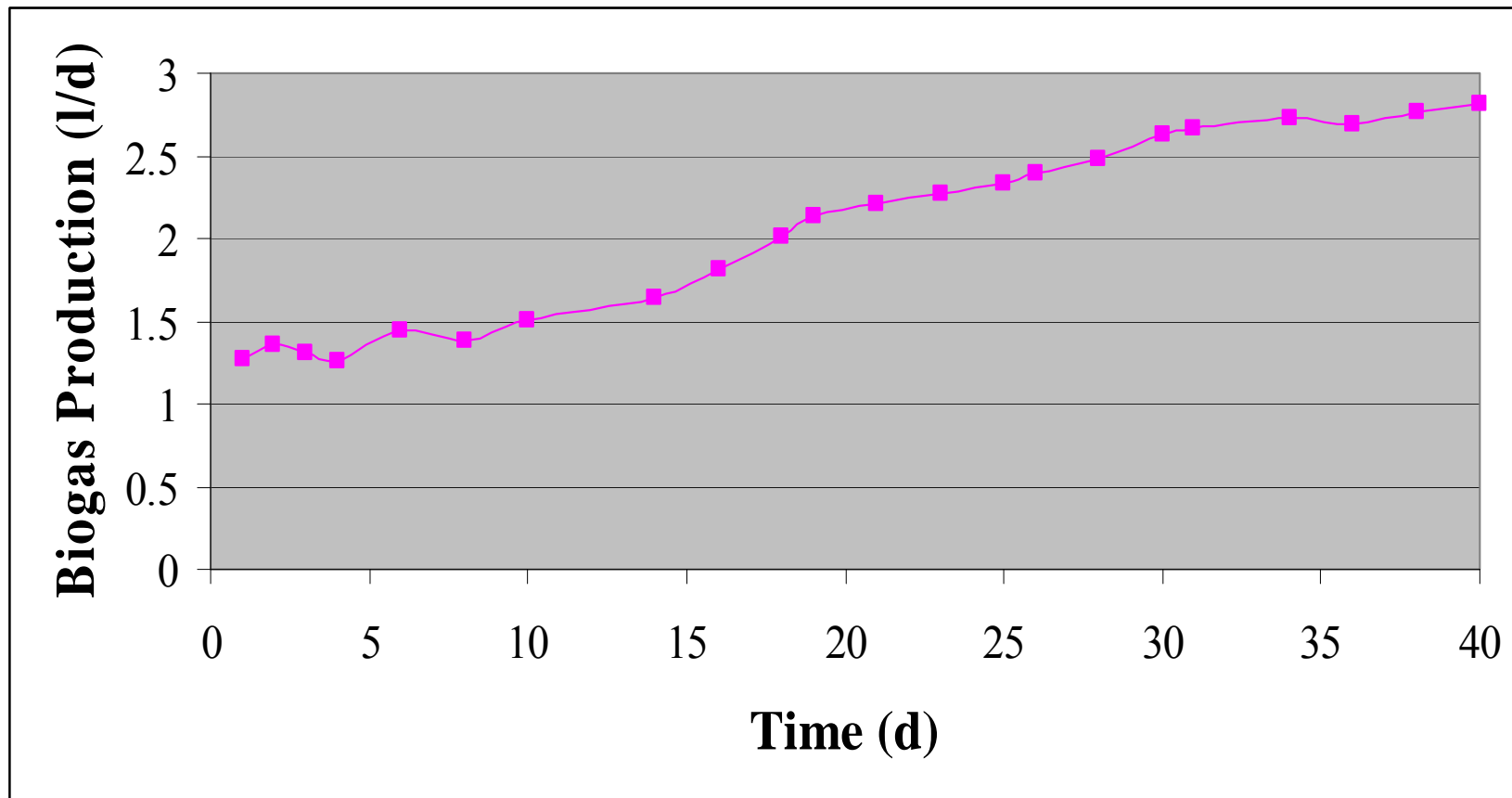


Figure 4.19 Biogas production of reactor J.

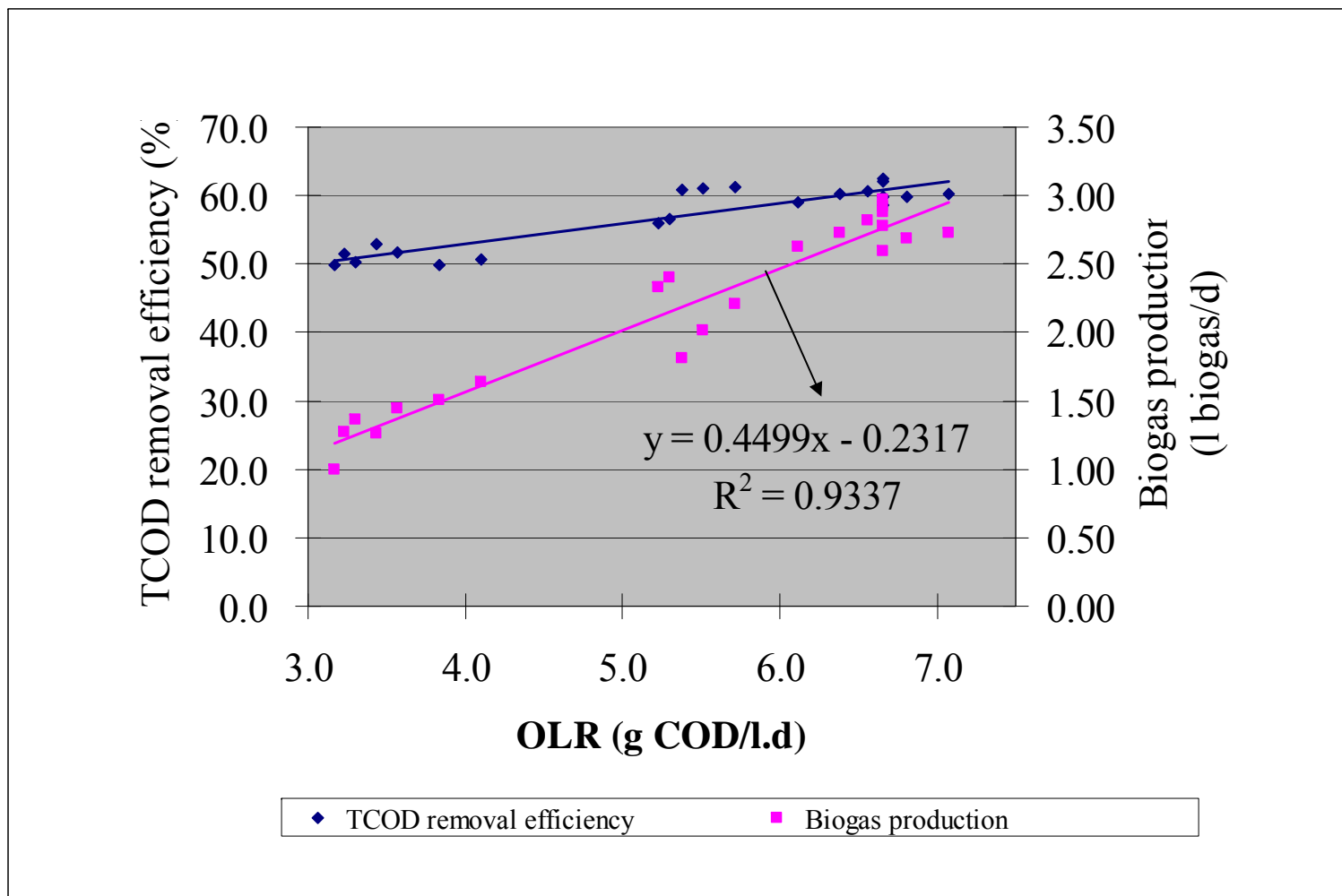


Figure 4.20 The effect of OLR on TCOD removal efficiency and biogas production of reactor J.

4.2.6. Investigation of the effect of HRT on co-digestion of primary sludge and whey with constant OLR

The purpose of the experiment was to investigate the effect of HRT on co-digestion of primary sludge and whey with constant OLR of 7.6 ± 0.1 g COD/l.d.

As discussed in 4.2.4, reactor G treated 100 ml primary sludge and 3.8 g whey powder per day with pH control ($\text{pH} = 6.9 \pm 0.1$). The OLR of reactor G was 7.6 ± 0.1 g COD/l.d and the HRT of reactor G was 20 days. In order to investigate the effect of HRT on the performance of reactors with same OLR as reactor G, reactor K with was set up, which treated 100 ml primary sludge and 100 ml whey solution (dissolving 3.8 g whey powder in 100 ml water, COD concentration was approximately 72 g COD/l) per day with pH control ($\text{pH} = 6.9 \pm 0.1$). The OLR of reactor K was similar to reactor G (7.6 ± 0.1 g COD/l.d), but the HRT of reactor K was 10 days.

Table 4.10 shows the performance comparison of reactor G and reactor K. Comparing the performance of reactor G and reactor K, the TCOD removal efficiency of reactor G ($72 \pm 2\%$) was 10% higher than that of reactor K ($62 \pm 2\%$), but the biogas production of reactor G (3.3 ± 0.2 l/d) was only slightly higher than that of reactor K (3.2 ± 0.2 l/d). The experimental results indicated that with similar OLR, decreasing the HRT from 20 days to 10 days, the biogas production were similar.

The hydraulic retention time (HRT) is the average time the liquid is held in the digestion process. Standard hydraulic retention time in a municipal sludge CSTR system without sludge recycle is 15-20 days (Water Pollution Control Federation, 1987). The biological solids retention time (SRT) is the average time the solids are held in the digestion process. For a CSTR system without recycle, $\text{SRT} = \text{HRT}$. The performance of a microbial community growing in an anaerobic digestion system is determined primarily by SRT (Water Pollution Control Federation, 1987). The three reactions (hydrolysis, fermentation, and methanogenesis) are directly related to SRT. In practice for CSTR sludge digestion, values for SRTs range from 10 to 20 days (WEF, 1998). In current study, by co-digestion of primary sludge and whey solution, the reactor could be operated successfully at HRT

/SRT of 10 days and at OLR of 7.6 ± 0.1 g COD/l.d with COD loading ratio of primary sludge to whey of 53 : 47.

Table 4.10 Comparison of reactor G and reactor K.

Reactor	G	K
Feed of primary sludge (ml)	100	100
Feed of whey	3.8 g powder	3.8 g + 100ml water
OLR (g COD/l.d)	7.6 ± 0.1	7.6 ± 0.1
COD loading ratio (PS:whey)	53:47	53:47
HRT (d)	20	10
TCOD concentration of influent (g COD/l)	152 ± 2	76 ± 2
TCOD concentration of effluent (g COD/l)	44 ± 2	28 ± 2
TCOD removal efficiency (%)	72 ± 2	62 ± 2
Biogas production (l/d)	3.3 ± 0.2	3.2 ± 0.2
pH control	Yes (6.9 ± 0.1)	Yes (6.9 ± 0.1)

4.2.7. The advantages of co-digestion primary sludge with whey

4.2.7.1. Primary sludge may serve as nutrient supplement and buffering reagent

As discussed before, anaerobic digestion of whey without pH control is not feasible (Ghaly, 1996). Supplemental alkalinity is required so as to avoid anaerobic process failure (Mockaitis et al., 2005). Addition of nutrients and trace element was also necessary to maintain the anaerobic treatment system.

The result of current study showed that with proper OLR and COD loading ratio of primary sludge to whey (70:30), it was feasible to anaerobic digest a mixture of primary sludge and whey without nutrient supplement and pH control. The primary sludge may serve as nutrient supplement and buffering reagent. For example, the OLR of reactor B was 3.8 ± 0.1 g COD/l.d, and the COD loading ratio of primary sludge to whey was approximately 70:30. The pH of reactor B ranged from 6.7 to 6.9 and the reactor was quite stable without pH control and nutrient supplement. For reactor F, the OLR was 5.8

± 0.1 g COD/l.d, and the COD loading ratio of primary sludge to whey was approximately 70:30. The pH of reactor F maintained above 6.6 and the reactor was quite stable without pH control and nutrient supplement. This was in agreement with Lo et al. (1988) studying the anaerobic digestion of a mixture of cheese whey and dairy manure. They reported that the stability of the AnRBC digesting a mixture of cheese whey and dairy manure was improved comparing to digestion of whey alone. The AnRBC could be operated successfully without the need to add any nutrient and buffering reagent to the mixture.

When the COD loading ratio of primary sludge to whey was approximately 30:70, the reactors appeared unstable without pH control, because of the deficient buffering reagent supplement. However, for the co-digestion reactors with pH control, the amount of sodium bicarbonate (NaHCO_3) used was much lower than digestion of whey alone. The highest sodium bicarbonate addition in current study was 1 g/l.d, while Kalyuzhnyi et al. (1997) supplemented up to 3 g/l.d sodium bicarbonate to increase the reactor buffer capacity, and Erguder et al. (2001) added 6 g/l.d sodium bicarbonate to the basal medium when anaerobic digestion of whey. Furthermore, no nutrients were supplemented for any of the reactors.

4.2.7.2. Increase OLR, biogas production and TCOD removal efficiency, decrease HRT

Table 4.11 shows the performance comparison of reactor A (digestion of primary sludge alone) and reactor K (co-digestion of primary sludge plus whey solution). Reactor A was fed with 100 ml primary sludge per day, while reactor K was fed with 100 ml primary sludge plus 100 ml whey solution (72 g COD/l) per day. The OLR and HRT of reactor A were 4.0 ± 0.1 g COD/l.d and 20 days, respectively. The OLR and HRT of reactor K were 7.6 ± 0.1 g COD/l.d and 10 days, respectively.

Table 4.11 Comparison of reactor A (digestion of primary sludge alone) to reactor K (co-digestion of primary sludge plus whey solution).

Reactor	A	K
Feed of primary sludge (ml)	100	100
Feed of whey	0	3.8 g + 100ml water
OLR (g COD/l.d)	4.0 ± 0.1	7.6 ± 0.1
COD loading ratio (PS : whey)	100:0	53:47
HRT (d)	20	10
TCOD concentration of influent (g COD/l)	80 ± 2	76 ± 2
TCOD concentration of effluent (g COD/l)	36 ± 2	28 ± 2
TCOD removal efficiency (%)	57 ± 3	62 ± 2
Biogas production (l/d)	1.7 ± 0.2	3.2 ± 0.2
pH control	No	Yes
pH	6.9 ± 0.1	6.9 ± 0.1

Comparing the performance of reactor A and reactor K, the biogas production of reactor K (3.2 ± 0.2 l/d) was 1.5 l/d higher than that of reactor A (1.7 ± 0.2 l/d). The influent TCOD concentration of reactor K (76 ± 2 g COD/l) was 4 g COD/l lower than that of reactor A (80 ± 2 g COD/l). The effluent TCOD concentration of reactor K (28 ± 2 g COD/l) was 8 g COD/l lower than that of reactor A (36 ± 2 g COD/l), which resulted in the TCOD removal efficiency of reactor K (62 ± 2 %) was 5% higher than that of reactor A (57 ± 3 %).

The results in Table 4.11 indicated that by co-digestion of primary sludge and additional whey solution, the OLR, the biogas production as well as the TCOD removal efficiency increased while the TCOD concentration of the effluent decreased comparing to digestion of the same amount of primary sludge alone. The reactor which only digested the primary sludge had the ability to digest more whey solution and produce more biogas without disturbing its original activity.

This was in agreement with the result of the study of Lo et al. (1988) investigating the anaerobic digestion of a mixture of cheese whey and dairy manure. They reported that the digestion of a mixture of cheese whey and screened dairy manure increased the methane production and COD reduction over that of the digestion of the screened dairy manure alone.

Generally, when digest primary sludge, hydrolysis is the rate limited step, while when digest whey, methanogenesis is the rate limited step because whey has very high biodegradability (about 99%) (Mockaitis et al., 2005). By co-digestion of primary sludge and whey, the system can be balanced and the performance of the reactor may be improved by increasing the OLR, biogas production and TCOD removal efficiency as well as decreasing the HRT and the TCOD concentration of the effluent.

This concept could be utilized to optimize the existing anaerobic digester in the wastewater treatment plant of Palmerston North City Council by means of co-digestion of primary sludge and whey solution, which can increase the biogas production of the digester and improve the effluent quality, at the same time, resolve the whey disposal problem of dairy industries.

CHAPTER 5 CONCLUSION

5.1 Semi-continuous whey digestion with pH control

The results of the semi-continuous whey digestion process demonstrated that without addition of nutrients, trace elements and heavy metals, the anaerobic digestion of whey failed at OLR of 3 g COD/l.d with pH control ($\text{pH} = 7.0 \pm 0.1$). The significant decrease of the TCOD and the SCOD of the reactor after the supplement of nutrients, trace elements and heavy metals indicated that nutrients, trace elements as well as heavy metals were necessary to maintain the anaerobic whey digestion system.

5.2 Anaerobic co-digestion of primary sludge and whey

During the investigation of anaerobic co-digestion of primary sludge and whey process, the performance of anaerobic digestion of primary sludge alone without pH control at HRT of 20 days was monitored as a control. The OLR of the reactor was 3.3 ± 0.1 g COD/l.d from day 1 to 7 and was 4.0 ± 0.1 g COD/l.d from day 8 due to the COD variation of the primary sludge. The biogas production was 1.3 ± 0.1 l/d from day 1 to 7 and was 1.7 ± 0.1 l/d from day 8. The TCOD removal efficiency was $51 \pm 3\%$ from day 1 to 7 and was $57 \pm 3\%$, from day 8.

A number of conclusions can be drawn from the study of the anaerobic co-digestion of primary sludge and whey process and these are summarized as follows:

- (1) The anaerobic co-digestion of primary sludge and whey process performed successfully without additional nutrients, trace elements and heavy metals.
- (2) When the COD loading ratio of primary sludge to whey was approximately 70:30, it was feasible to co-digest primary sludge and whey without pH control. The primary sludge served as buffering reagent in the co-digestion system.

(3) When the COD loading ratio of primary sludge to whey was approximately 30:70, addition of buffering reagent (sodium bicarbonate: NaHCO_3) was necessary to maintain the stability of the co-digestion system. However, the amount of sodium bicarbonate supplement was approximately 2 g/l.d less than digestion of whey alone.

(4) Digestion of the mixture of primary sludge and whey with higher whey content achieved higher biogas production and TCOD removal efficiency. With same OLR (3.3 ± 0.2 g COD/l.d) at HRT of 20 days, the biogas production and TCOD removal efficiency were 1.5 ± 0.1 l/d and 54 ± 3 %, respectively, when the COD loading ratio of primary sludge to whey was approximately 70:30; while the biogas production and TCOD removal efficiency were 1.8 ± 0.1 l/d and 57 ± 3 %, respectively, when the COD loading ratio of primary sludge to whey was approximately 30:70.

(5) Without pH control, the OLR of the anaerobic co-digestion reactor could reach 5.8 ± 0.1 g COD/l.d at COD loading ratio of primary sludge to whey of 70:30 and HRT of 20 days. The biogas production of the reactor was 2.5 ± 0.1 l/d and the TCOD removal efficiency was 68 ± 3 %. Comparing to the performance of the reactor treating same amount of primary sludge alone (OLR = 4.0 ± 0.1 g COD/l.d, HRT = 20 days), the biogas production and the TCOD removal efficiency of the co-digestion reactor increased by 0.8 l/d and 11%, respectively.

(6) By controlling the pH at 6.9 ± 0.1 , the OLR of the co-digestion reactor could reach 8.1 ± 0.1 g COD/l.d at COD loading ratio of primary sludge to whey of 49:51 and HRT of 20 days. The biogas production of the reactor was 3.5 ± 0.1 l/d and the TCOD removal efficiency was 77 ± 1 %. Comparing to the performance of the reactor treating same amount of primary sludge alone (OLR = 4.0 ± 0.1 g COD/l.d, HRT = 20 days), the biogas production and the TCOD removal efficiency of the co-digestion reactor increased by 1.8 l/d and 20%, respectively.

(7) The biogas production and the TCOD removal efficiency of the co-digestion system increased with the increase of OLR (from 3.0 to 7.0 g COD/l.d). The positive linear

relationship between the OLR and the biogas production for the 2-litre reactor can be expressed by the equation: $y = 0.4499x - 0.2317$, with correlation coefficients (R^2) equal to 0.9337, where y is biogas production (l/d) and x is OLR (g COD/l.d).

(8) The co-digestion reactor could be operated successfully at HRT of 10 days. Decreasing the HRT from 20 days to 10 days at similar OLR of 7.6 ± 0.1 g COD/l.d, the biogas production were similar (3.3 ± 0.1 l/d and 3.2 ± 0.1 l/d, respectively).

(9) The performance comparison between digestion of primary sludge alone and co-digestion of same amount of primary sludge plus additional whey solution demonstrated that the anaerobic primary sludge digester had the capability treating additional whey solution. The co-digestion process with OLR of 7.6 ± 0.1 g COD/l.d (COD loading ratio of primary sludge to whey was approximately 53:47) at HRT of 10 days resulted in 1.5 l/d more biogas production and 5 % higher TCOD removal comparing to digestion of primary sludge alone at OLR of 4.0 ± 0.1 g COD/l.d and HRT of 20 days. The anaerobic co-digestion of primary sludge and whey proved to be a suitable technology for optimisation of the existing anaerobic primary sludge digesters in the wastewater treatment plant in terms of increasing OLR and biogas production as well as resolving the whey disposal problems for dairy industries.

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