# PERFORMANCE EVALUATION AND MODELING OF UPFLOW ANAEROBIC SLUDGE BLANKET PROCESS TREATING DAIRY WASTEWATER

**Mr.Kraichat Tantrakarnapa**

**A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of**

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# การประเมินและจัดทำระบบแบบจำลองกระบวนการชั้นตะกอนแอนแอโรบิคแบบไหลขึ้นในการบำบัดน้ำเสียจากโรงงานนมและผลิตภัณฑ์นม

นายไกรชาติ ตันตระการอาภา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรดุษฎีบัณฑิต

สาขาวิชาวิศวกรรมสิ่งแวดล้อม

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ไกรชาติ ตันตระการอาภา : การประเมินและจัดทำระบบแบบจำลองกระบวนการชั้นตะกอนแอนแอโรบิคแบบไหลขึ้นสำหรับบำบัดน้ำเสียจากโรงงานนมและผลิตภัณฑ์นม
(PERFORMANCE EVALUATION AND MODELING OF AN UPFLOW ANAEROBIC SLUDGE BLANKET PROCESS TREATING DAIRY WASTEWATER), อ.ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ จงจินต์ ผลประเสริฐ, Ph.D. 171 หน้า. ISBN 974-533-261-5

การศึกษาการบำบัดน้ำทิ้งจากโรงงานนมและผลิตภัณฑ์นมดำเนินการที่อุณหภูมิบรรยากาศโดยการใช้ กระบวนการชั้นตะกอนแอนแอโรบิคแบบไหลขึ้น (UASB) น้ำเข้าระบบมีค่า COD เฉลี่ย 938 mg/L ทดลองที่ 4 ระยะเวลากักเก็บ 12-24 ชั่วโมง เทียบเท่ากับอัตราภาระบรรทุกอินทรีย์ 1.01-2.07 kg COD/$m^3$-d เมื่อเข้าสู่สภาวะสมดุลซึ่งใช้ระยะเวลาประมาณ 3 เดือน มีการเก็บตัวอย่างและวิเคราะห์ คุณภาพน้ำเข้าและออกจากระบบเพื่อศึกษาประสิทธิภาพการบำบัด พบว่ามีประสิทธิภาพในการบำบัดดังนี้ COD = 66-92%, BOD = 90-97%, TS = 58-82%, TDS = 48-77%, อินทรีย์ไนโตรเจน = 65-83%, และฟอสฟอรัสอินทรีย์ = 68-95% โดยภาพรวมพบว่าระบบ UASB สามารถใช้บำบัดน้ำเสียจากโรงงานนมและผลิตภัณฑ์นมขั้นแรกได้ดี และมีก๊าซชีวภาพเป็นผลพลอยได้ ในอัตรา 552 ลิตรต่อกิโลกรัมของ COD ที่ใช้ไป ไม่พบว่าการเปลี่ยนแปลงอัตราการไหลขึ้นของน้ำเข้าระบบจะมีผลต่อขนาดของเม็ดตะกอนอย่างมีนัยสำคัญทางสถิติ ในการศึกษาการแตกตัวของกรดพบว่าในน้ำที่ระบายออกจากระบบมีปริมาณกรดแลคติกและโพรไพโอนิกสูง อัตราการเกิดปฏิกิริยาการแตกตัวของกรดอะเซติกไปเป็นก๊าซมีเทนจะเกิดช้าหรือเป็นข้อจำกัดของกระบวนการย่อยแบบแอนแอโรบิค ค่าจลน์ศาสตร์ของระบบพบว่ามีค่า k, $K_s$ และ Y เป็น 13 $d^{-1}$, 14.73 mg/L and 0.19 g VSS/g COD ตามลำดับ การประมาณการเกิดก๊าซชีวภาพโดยใช้แบบจำลองคอมพิวเตอร์ที่สร้างขึ้นพบว่า ค่าที่ประมาณสอดคล้องกับผลการวิเคราะห์ที่ตรวจวัดได้ในห้องปฏิบัติการโดยการสอบเทียบการวิเคราะห์ความไวของแบบจำลองพบว่า ค่า $K_S$ มีค่าความไวมากกว่า ค่า $\mu_m$

สาขาวิศวกรรมสิ่งแวดล้อม ลายมือชื่อนักศึกษา.....

ปีการศึกษา 2546 ลายมือชื่ออาจารย์ที่ปรึกษา.....

KRAICHAT TANTRAKARNAPA: PERFORMANCE EVALUATION AND MODELING OF UPFLOW ANAEROBIC SLUDGE BLANKET PROCESS TREATING DAIRY WASTEWATER, THESIS ADVISOR: CHONGCHIN POLPRASERT, Ph.D. 171 PP. ISBN: 974-533-261-5

ACID DISTRIBUTION/ ANAEROBIC WASTEWATER TREATMENT/ BIOGAS PRODUCTION/ GRANULE SIZE/ UASB

Investigation on the dairy wastewater treatment were undertaken at ambient temperature in 16.8 L effective volume laboratory-scale upflow anaerobic sludge blanket (UASB) reactor receiving the average influent COD of 938 mg/L for 15 months of 4 HRTs. The feeds of the synthetic dairy wastewater were operated with the 4 Hydraulic Retention Times (HRT) ranging between 12 – 24 h, and at the equivalent to the organic loading rates of 1.01 – 2.07 kg COD/$m^3$-d. After the steady-state condition was reached, which took about 3 months, the effluent quality parameters were sampled and analyzed to quantify the treatment efficiencies. The following efficiencies could be observed- COD = 66-92%, BOD = 90-97%, TS = 58-82%, TDS = 48-77%, organic nitrogen = 65-83%, and organic phosphorus = 68-95%. The average biogas production rate was 552 L per kilogram COD removed with 375 L of methane gas. The granule size was insignificant different for various upflow velocities ($\alpha$=0.05). Major acids found in the effluent were lactic and propionic acids. The reaction rate of acid distribution of acetic acid to methane gas was determined and found to be the limiting step of biogas production. The kinetic coefficients k, $K_s$,

and Y were 13 $d^{-1}$, 14.73 mg/L and 0.19 g VSS/g COD, respectively. The sensitivity analysis was also performed and found that it was more sensitive to $K_s$ value than $\mu_m$. The estimation of biogas production by using constructed computer simulation model indicated that the estimated values agreed with the experimental results. The overall result obtained from this study indicated that the UASB can be used as the biogas production process and the treatment unit for dairy wastewater treatment, as well.

School of Environmental Engineering Student Signature……..…….……..

Academic year 2003 Thesis Advisor Signature……..…….…..

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Kraichat Tantrakarnapa

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# List of Symbols and Abbreviations

| | | |
|---|---|---|
| APHA | = | American Public Health Association |
| B | = | Contois kinetic parameter |
| BOD | = | Biochemical Oxygen Demand |
| cm | = | Centimeter |
| CIP | = | Cleaning in place |
| COD | = | Chemical Oxygen Demand |
| CSTR | = | Single Continuous Stirred Tank Reactor |
| $^{o}C$ | = | Degree Celsius |
| d | = | Dispersion coefficient |
| D.F.P.O | = | Dairy Farming Promotion Organization |
| D/μL | = | Dispersion number |
| DIW | = | Department of Industrial Works |
| F/M | = | Food to microorganism ratio |
| g | = | Gram |
| g/L | = | Gram per liter |
| g VSS/L | = | Gram Volatile suspended solids per liter |
| GSS | = | Gas-Solids Separator |
| GLS | = | Gas-Liquid-Solids separator |
| h | = | Hour |

# List of Symbols and Abbreviations (continued)

| | | |
|---|---|---|
| HRT | = | Hydraulic retention time |
| HTST | = | High Temperature Short Time |
| kg/d | = | Kilogram per day |
| Kg DS/$m^3$ | = | Kilogram dry solids per cubic meter |
| $K_s$ | = | Saturation constant |
| $K_d$ | = | Death rate constant |
| LTLT | = | Low Temperature Long Time |
| L | = | Liter |
| mm | = | Millimeter |
| mL | = | Milliliter |
| $m^3$/d | = | Cubic meter per day |
| $m^3$/kg | = | Cubic meter per kilogram |
| mg/L | = | Milligram per liter |
| MIT | = | Massachusetts Institute of Technology |
| MLVSS | = | Mixed Liquor Volatile Suspended Solid |
| ND | = | Not detectable |
| NLR | = | Nitrogen Loading Rate |
| OLR | = | Organic Loading Rate |
| ppm | = | Part per million |
| PCE | = | Perchloroethylene |
| PLR | = | Phosphorus Loading Rate |

# List of Symbols and Abbreviations (continued)

| | | |
|---|---|---|
| r | = | Reaction rate |
| $S_o$ | = | Substrate concentration in feed |
| S | = | Substrate concentration in effluent |
| TBE | = | Tick-borne encephalitis |
| TCE | = | Trichloroethene |
| TDS | = | Total Dissolved Solids |
| TKN | = | Total kjeldahl nitrogen |
| TP | = | Total phosphorus |
| $T_{mean}$ | = | Average retention time |
| TS | = | Total Suspended Solids |
| UASB | = | Upflow Anaerobic Sludge Blanket |
| UHT | = | Ultra High Temperature |
| VFA | = | Volatile Fatty Acid |
| VSS | = | Volatile suspended solids |
| VOL | = | Volumetric Organic Loading |
| V | = | Volume of reactor |
| VDS | = | Volatile Dissolved Solids |
| $X_o$ | = | Concentration of biomass in the feed |
| X | = | Concentration of biomass in the reactor |
| Y | = | Yield coefficient |
| $\mu$ | = | Specific growth rate |

# List of Symbols and Abbreviations (continued)

| | | |
|---|---|---|
| $\mu_m$ | = | Maximum specific growth rate |
| $\sigma^2$ | = | Coefficient of variation |

# Chapter I

# Introduction

## 1.1 Statement of Problem

Currently, demand for dairy products in Thailand has been increasing. In 2001, there were approximately 88 dairy plants in Thailand (Department of Industrial Works, 2002) and most of them did not have wastewater treatment system, particularly the small-scale dairy plants. Wastewater is a significant problem for dairy plant operation since a large quantity of water is used for product addition and utensil cleaning. In the processing of milk in Thailand, 4-11 and 3-6 liters of water are used per liter of milk processed for the production of less than 50 tons/day and a large-scale plant (>50 tons/day), respectively (DIW, 2002). Subsequently, approximately 80% of used water is discharged as wastewater, which contains a large amount of milk constituents such as casein, lactose, fat and others. Panesar et al. (1999) reported that 6-10 liters of wastewater were generated per liter of milk product. All these contribute towards vary high concentrations of biochemical oxygen demand (BOD) and nutrients contained in dairy wastewater, which are the main causes of the deterioration of the quality of receiving water bodies. Most of dairy plants in Thailand, particularly small-scale ones, discharge wastewater directly to nearby areas such as idle land and/or natural receiving water body. The discharged volume of wastewater depends on the size of plant and their activities. Therefore, the plants located in the urban area may confront with the land lacking and/or the high cost of land if they use the method of

wastewater treatment that require a large area of land. The treatment of dairy wastewater with less area requirement should be appropriate. The popular method of dairy wastewater treatment in Thailand is using aerator and air blower, which require energy of 1.5 – 2.5 kW-h per kilogram of BOD removed. Due to the high content of organic matter in dairy wastewater that can be converted to gas under anaerobic conditions, it could be used as energy source for various purposes such as electricity generation or using for heat generation if high volume of biogas in terms of methane was formed. The feasibility of its usage depends on the content of methane in biogas generated and other related factors. Other factors to be considered include those of the economics and engineering. Thus, an anaerobic process can be used not only to treat wastewater but also to obtain the energy by means of waste recovery. Jence, the benefits of this approach will be the protection of the environment and the recovery of energy source.

During the past few years, efforts have been made to develop technologies for the treatment of dairy wastewater. However, many of these technologies are economically non-viable for the small dairy plants due to high capital costs (Panesar et al., 1999). Various attempts have been also made to alleviate the problems with available technologies for treating the dairy wastewater of small-scale and medium-scale dairy plants.

At present, many anaerobic treatment processes are available. The most interesting one among them is the upflow anaerobic sludge blanket (UASB) process. UASB process uses suspended growth biomass, but the gas-liquid-solids separation system is integral with the bioreactor. Influent wastewater enters the bottom of the bioreactor through a distribution system. Dense slurry of granule forms in the lower

portion of the bioreactor, and the combined effects of the influent wastewater distribution and gas production result in mixing of the influent wastewater with the granules. The main advantage of this process is that it does not use material to be harbored by microorganisms and concentrated biomass is retained in the reactor. Furthermore, it requires low energy input because the anaerobic microorganisms do not use oxygen, thus aerator is not necessary to be installed. Therefore, the cost of construction and operation will be reduced since the supporting materials are not required, oxygen generator is not used, and less area is required. The operational performance is high without sludge feeding since the granular sludge remained in the reactor for a long period and it has no problem of excess sludge to be disposed of. Moreover, this process also has a by-product methane gas that can be used as energy source.

The anaerobic process is a highly complex system characterized by various influencing factors. In the past decades, intensive development in computer technology has brought new possibilities in wastewater treatment process design, prediction tool and plant operation. Mathematical models and simulation programs are powerful tools for improvement of design and operation of new plants prior to actual construction and operation. They give the opportunity to gain insight into the behavior of wastewater treatment plants under various conditions, and to elaborate different design strategies.

As mentioned above, UASB wastewater treatment process for treating dairy wastewater had never been used in Thailand for treating dairy wastewater. Therefore, this study emphasized on the use of this process to determine the feasibility or process performance. Furthermore, the high concentration of BOD in dairy wastewater will be

benefit to the dairy plant itself in terms of energy recovery particularly small-scale plants that depended upon the out site energy. Normally, they used energy for milk production from various sources such as diesel oil, liquid petroleum gas or others. If the recovered energy was sufficient volume, it would be used as energy source instead of the above mentioned sources. UASB is the one type of anaerobic process that consists of 3 phases namely hydrolysis, acidogenesis, and methanogenesis. Some organic acids occur in acidogenesis and finally converted to acetic acid and methane gas. If organic acids were found in the effluent, it indicated the performance of reactor and the methane production. Acid distribution in reactor can be used to determine the reaction rate of anaerobic process. There was also no studies on this issue in Thailand.

The expected results obtained from this study are not only to evaluate the performance of UASB process for treating dairy wastewater that will be benefits to overall environment but also obtaining the energy as by-products. In addition, the reaction rate determination can be used to indicate the limiting step of anaerobic process by using UASB. This will be benefit for designing to achieve the requirement for example, the removal efficiency and methane production. The obtained kinetics coefficients of UASB treating dairy wastewater can be used as model input. The developed model by using STELLA software can be used for further design in pilot scale or actual plant design for predicting the methane formation.

## 1.2 Objectives

The overall objective of this study was to evaluate the performance of UASB wastewater process for treating dairy wastewater and to develop the simulation model

for methane formation. Specific objectives can be categorized into 2 groups as follows.

### 1.2.1 Performance Evaluation

1) To gain in-depth knowledge about the behavior of anaerobic bacteria used to treat dairy wastewater.

2) To study the influence of engineering factors namely Y, k and $K_s$.

3) To study the acid distribution obtained from UASB process for dairy wastewater treatment.

4) To determine the reaction rate in anaerobic process for treating dairy wastewater by using UASB process.

### 1.2.2 Simulation Model Development

1) To develop a mathematical model by using STELLA software.

2) To predict methane production from UASB process treating dairy wastewater and effluent concentration.

## 1.3 Scope of the Study

A laboratory-scale UASB reactor was constructed, and fed with synthetic dairy wastewater under ambient temperature at the Environmental Laboratory of the Department of Environmental Health Science, Faculty of Public Health, Mahidol University. The hydraulic retention time was varied as 12, 16, 20 and 24 hrs, corresponding to four different influent flow rates of 33.6, 25.2, 20.16 and 16.8 L/d, respectively. The parameters of pH, temperature, COD, $BOD_5$, SS, VSS, TP, ortho phosphate, TKN, nitrate nitrogen, ammonia nitrogen and organic nitrogen regarding to reactor performance, were determined according to the "Standard Methods" (APHA et

al., 1998). The nutrient removal, kinetics parameters, granular size, methane production, and various types of organic acids were analyzed statistically in the forms of correlative equations. The efficiency of dairy wastewater treatment using UASB process was estimated by means of experimental results compared with those predicted by computer simulation. Mathematical model for methane formation in an UASB process for treating dairy wastewater was developed, using STELLA software. The experimental results were used for model calibration, validation, and sensitivity analysis.

# Chapter II

# Theoretical Concepts and Literature Reviews

## 2.1 Dairy Plant in Thailand

Since Thailand is an agriculture-based country, dairy industry is increasing gradually. In 2001, there were approximately 88 dairy plants in Thailand (Department of Industrial Works, 2002) categorized into 3 groups based on the production capacity as shown in Table 2.1.

**Table 2.1** Criteria for size category of dairy plant in Thailand.

| Industrial scale | Production capacity |
|---|---|
| Small scale | 0.1 – 10 tons /d |
| Medium scale | 10 – 50 tons/d |
| Large scale | > 50 tons/d |

Source: http://www2.diw.go.th/ctu

In the future, it is expected that the number of dairy plants will be further increasing since Thai people are becoming more familiar with consumption of milk and other dairy products. Therefore, due to the increasing milk demand in Thailand, the potential impact on the environment from the dairy plants should be under surveillance.

Dairy cows are raised in all regions of Thailand. There are 173 milk collection

centers collecting milk from farmers. In the collection centers, there are cooling facilities to maintain a good quality of milk before delivery to plant. Milk is also processed in some collection centers. The milk centers can be classified into 4 types as follows.

**2.1.1 Government Milk Collection Centers:** Such centers belong to the Dairy Farming Promotion Organization (D.F.P.O) located in 4 regions: central-Muaklek district, Saraburi province; southern-Prachuabkhirikhan province; northern-Chiangmai province; and northeastern-Khon Kaen province.

**2.1.2 Cooperatives Milk Collection Centers:** There are 62 cooperatives milk collection centers collecting fresh milk from their members. Most of the cooperatives only collect fresh milk and deliver to dairy plants. Only 9 cooperatives, not only collect, but also process fresh milk.

**2.1.3 Academic Institutes:** There are 53 academic institutes, which comprise of universities and agricultural colleges collecting fresh milk from their own dairy farms, farmers, or other sources**.** These institutes usually process their milk themselves.

**2.1.4 Others:** Some milk collection centers belong to private dairy farmers groups and companies. These centers collect fresh milk from their own cows or from members. Some centers only collect the fresh milk and deliver to dairy plants, while some have the processing plant.

## 2.2 Dairy Processing

### 2.2.1 Dairy Process

In general, there are 3 types of dairy products in Thailand namely

Pasteurized milk, ultra high temperature (UHT ) milk, and yogurt and drinking yogurt. Raw milk is processed in accordance with the following three steps, prior to any of the three dairy products production.

1) Raw milk reception process: Some industries own their dairy farms to produce raw milk; thus they have to mix their own milk with others if their milk is not sufficient for dairy products production. Raw milk from all sources is inspected prior to mixing. The parameters to be checked are as follows: an odor, cleanliness, contamination, bacterial concentration, and fat quantity. Normally, there are two types of reception processes, the first is milk container reception, and the second is the milk container truck reception.

2) Raw milk storage: Accepted raw milk from reception unit is fed into provided storage tanks, which must be maintained at 6-8$^{o}$C temperature. In case of the milk temperature exceeding the designed temperature, it will be sent to cooling plate or cooling tank for temperature reduction.

3) Thermization: This unit is used, in some plants, to reduce the quantity of microorganisms by using high temperature of 75$^{o}$C for 16 seconds. Three kinds of milk products produced in dairy plant are: pasteurized milk, UHT milk, and yogurt.

Pasteurized Milk: The raw milk is withdrawn from the tank and some imported milk powder are also used. Raw milk is fed to filter clarifier prior to pasteurization in order to separate contaminated materials. In some factories, the thermization is included in this step for bacteria reduction to extend the milk life.

Pasteurization is the process that heats milk to a certain temperature and then chills it, in order to kill harmful bacteria. Normally, pasteurization can be

grouped into 2 types as follows: (1) Batch Pasteurization: this system is practical for small-scale plants with the temperature range of 62.8-65.6$^{o}$C for 30 minutes. Then, the temperature is decreased to 10$^{o}$C. This system is suitable for milk volumes of 1,500-2,000 liter, and (2) Continuous Pasteurization: There are two types of this system, the first is Low Temperature-Long Time (LTLT) system in which milk is heated to temperatures ranging 62.8-65.6$^{o}$C for 30 minutes. The second system is High Temperature Short Time (HTST) system that heats milk at 72$^{o}$C for 15 seconds and later keeps it at 5$^{o}$C or lower temperature.

After this step, milk is passed through a separator to eliminate contaminants and sent to standardizer. Standardization is a step to adjust fat content. There are 3 methods of standardization described as: 1) pre-standardization: for this method, milk is standardized prior to pasteurization. If fat content is lower than acceptable level, cream will be added. This approach requires a large milk tank for mixing, 2) post-standardization: raw milk is standardized after pasteurization. It is very sensitive since pasteurized milk may be infected with bacteria, and 3) standardization in line: currently, this method is very popular since it can solve the problems of two previous methods by increasing the capacity of a separator.

After standardization, standardized milk is passed through homogenizer to treat milk so that the particles of fat are broken down and the cream is blended with the rest. From this step, processed milk is stored in the specific milk tank prior to filling process. At this stage, flavored milk can be established and distributed to each filler. Normally, milk containers are plastic bags, paper boxes and bottles. This processed milk is kept in cold storage area.

UHT milk: UHT milk process was developed by Jonas Nielson in 1913

by using high temperature in the range of 140-150$^{o}$C for 2 seconds. These temperature range and time period do not change the milk properties; except making it free of bacteria. The method of heating process can be grouped into 2 types; they are 1) direct Heating: Steam is directly used to mix with raw milk by means of steam injection or milk injection to steam tank, so called steam infusion. Heating milk to 75$^{o}$C is the first step and then conveyed to the container having temperature of 140-150$^{o}$C and pressure of 460 kPa for approximately 2 seconds. Normally, this method is used for high volume production (more than 20,000 liters/hour), and 2) indirect Heating: Firstly, raw milk is heated to 66$^{o}$C and then conveyed to homogenizer and heated more to 138$^{o}$C. After the heating, it was rapidly cooled to 76$^{o}$C by using pasteurization and 20$^{o}$C by cold water, respectively, prior to aseptic packing process.

In this method, pasteurized milk is sent to UHT sterilizer, which includes homogenization. Then sterilized milk is ready for filling process and can be kept at ambient conditions.

Yogurt: In general, yogurt is slightly sour thick liquid food, consisting of milk fermented by added bacteria and often flavored with fruit. Two types of popular yogurt are set yogurt and drinking yogurt. Quality of yogurt depends upon the following aspects: raw milk quality, milk additives, homogenization, heating, and bacteria preparation.

The pasteurized milk is cooled to 45$^{o}$C and added with seed culture starter and kept in designed temperature tank of 42-43$^{o}$C. If the pH (4.2-4.5) of milk is achieved at the design criteria, it is cooled down to 18-20$^{o}$C. The overall Process of milk production is illustrated in Figure 2.1.

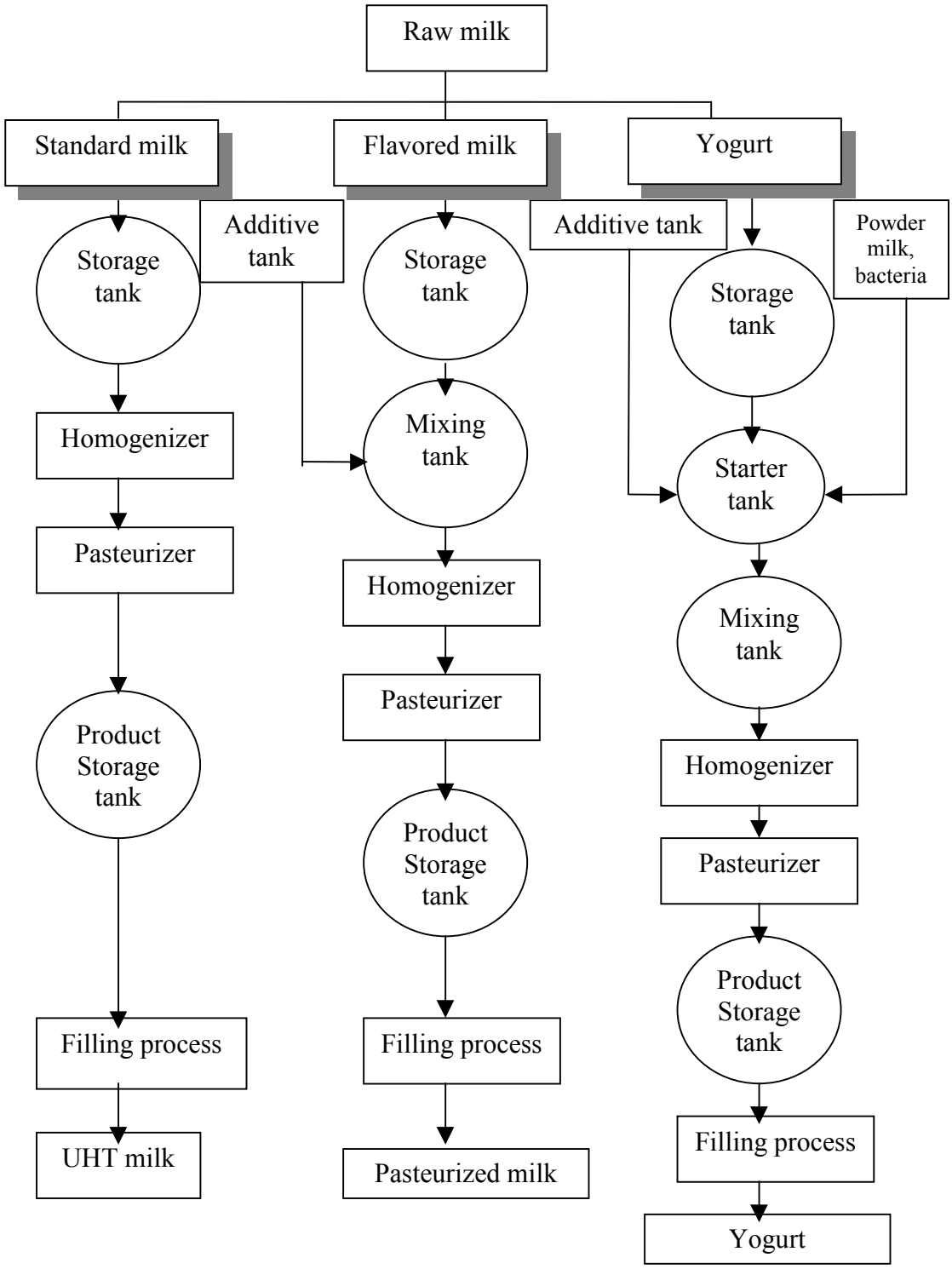



**Figure 2.1** Process of milk products production (ready to drink).

### 2.2.2 Characteristics of Dairy Wastewater

Dairy wastewater contains a large amount of the milk constituents such as casein, lactose, fat, and inorganic salts, besides detergents and sanitizing agents used for washing (Panesar et al., 1999). All these contribute to high concentrations of biochemical oxygen demand (BOD) and chemical oxygen demand (COD). The effluents from the milk processing industries originate from following sources, namely, washing of churns and process equipment, processing losses, e.g., butter washing in evaporators and deliberate wastage of low value products e.g., whey. The generated wastewater is largely organic-containing in nature and essentially consists of a solution of milk, milk products and cleaning materials. The quantity and quality of dairy wastewater vary from plant to plant. However, the characteristics of dairy wastewater related to cleaning and processing operation can be classified into 8 groups as described below (Alvarez et al., 1998).

1) The washing and cleaning out of products remaining in tanks of the trucks, piping, and other equipment (performed routinely after every processing cycle).

2) Spillage produced by leaks, overflow, freezing-on, boiling-over, equipment malfunction, or careless handling.

3) Processing losses, including: sludge discharged from CIP (Cleaning in place) clarifiers, product wasted during HTST step of pasteurization, start-up, shut-down, and product change-over, discharges from bottle and case washers, and splashing and container breakage in automatic change-over in filling machines.

4) Waste of spoiled products, returned products, or by-products

such as whey.

5) Detergents and other compounds used in the washing and sanitizing solutions that are discharged as waste.

6) Leakage of lubricants from conveyors, stackers, and other equipment from cleaning operations.

7) Routine operation of toilets, washrooms, and restaurant facilities at the plant.

8) Waste constituents that ultimately go to wastewater.

Rajeshwari et al. (2000) mentioned that liquid waste in a dairy plant originates from manufacturing process, utilities and service sections. The various sources of waste generation were from milk cans, equipment, bottles and floor washing. The dairy wastewater analyzed in some Asian countries like India (Panesar et al., 1999) showed $BOD_5$ and COD to be in the range of 390-582 mg/L and 964-1,270 mg/L, respectively in Panjab State.

Generally, dairy wastewater pH varies from day to day based on plant activity. Wastewater from the overall plant has an average pH of 7.6 with the range of 2.0 – 12.5. The characteristics of wastewater generated from various sources of dairy plant are illustrated in Table 2.2. The ratio of BOD and COD of dairy wastewater varies, depending on the types of dairy products. Normally, the range of BOD: COD ratio is 0.11-0.80 with the average of 0.53 (Usakorn, 1992).

## 2.3 Treatment Technologies

Because of high-strength pollution from dairy wastewater, the reduction of pollution can be achieved by several treatment methods, prior to discharge. The

**Table 2.2** Characteristics of dairy wastewater generated from various sources.

| Parameter | Concentration (mg/L) | | | |
|---|---|---|---|---|
| | I* | II* | III* | IV** |
| BOD | 500-1,000 | 40-48,000 | 90-12,000 | 600 – 960 |
| COD | 1,000-15,000 | 80-95,000 | 180-23,000 | 854 – 1464 |
| SS | 200-3,000 | 24-4,500 | 7-7,200 | 95 - 890 |
| TKN | 16-43 | 1-80 | 1-70 | 12 - 25 |
| Ammonia nitrogen | - | - | - | - |
| TP | 15-23 | 9-120 | 4-150 | 0.2 – 0.26 |
| Oil | - | 35-500 | 0-2,100 | - |
| pH | 3-11 | 4.4-9.4 | 3-13.2 | 6.5 - 13 |
| Temperature | 28-35 | 18-55 | 11-72 | 11-72 |
| DO | 0-0.5 | - | - | - |

Note: I = dairy wastewater from Kasetsart University dairy plant.

II = dairy wastewater from dairy plant in U.S.A.

III = dairy wastewater from dairy plant in New Zealand.

IV = dairy wastewater from Suranaree University of Technology dairy plant samples were taken on 27, 30 September, 4 October and 2 Novermber 1999.

Source: *Usakorn (1992), **Lawanwattanakul (2002)

methods employed in the treatment of dairy wastewater constitute a variety of physical, chemical and biological processes. Some of the previous studies of dairy wastewater treatment reported by Panesar et al. (1999) can be summarized as below.

### 2.3.1 Aerobic Treatment Processes

1) Activated Sludge Process: The activated-sludge process results in an aerobic degradation of the organic matter in the dairy wastewater. The treatment of

dairy effluent using this method has been reported to achieve 87-89% of BOD reduction. A maximum of 61.5% COD removal was observed at a F/M ratio of 0.039 per day by the activated-sludge process. Although this system offers high BOD and COD reduction, it can not be successfully used for the treatment of industrial wastewater in India due to high capital investment, operation costs, continuous supervision and the performance drops at low temperature.

2) Oxidation Ditch: Oxidation ditch is another form of activated-sludge process used for the treatment of dairy wastewater. The application of oxidation ditch for the treatment of dairy wastewater in India was reported with the BOD reduction from 910 to 30 mg/L. However, the operation failed to function at low temperature due to cessation of microbial activity (Panesar et al., 1999).

3) Rotary Biological Contractors: The use of rotating biological contractor (RBC) for the treatment of dairy wastewater appears to be encouraging due to its low energy consumption and ease of operation and maintenance. A BOD removal of 98% in dairy effluent by this method has been recorded. The maximum removal of COD achieved by this method at hydraulic loading rate of 0.08 $m^3/m^2$-d was 89.9% Panesar et al. (1999).

4) Trickling Filters: The wastewater flowed over the microbial growth attached to the mixed medium consisting of a bed or rocks, slag, plastic filter or high molecular weight synthetic resin in tricking filters for the treatment of dairy wastewater. The reduction in BOD and COD from 300 to 10 ppm and 150 to 11 ppm, respectively, were obtained Panesar et al. (1999).

5) Aerobic Lagoons: Aerobic lagoons are used in many countries for treatment of dairy waste to store effluents during winter for subsequent spray

irrigation. The aerobic lagoons were used for the complete treatment of dairy wastewater in Czechoslovakia with 95% BOD removal. This process has been reported to be uneconomical as the temperature had to be maintained at 35-37$^{o}$C and the operation requires much land.

### 2.3.2 Anaerobic Treatment Processes

The term, anaerobic process, refers to a diverse array of biological systems from which dissolved oxygen and nitrate-N are excluded. In most instances, they are operated to convert biodegradable organic matter, both soluble and particulate, to methane and carbon dioxide. Anaerobic processes have been used in wastewater treatment systems for more than a century; initially to stabilize the solids produced.

1) Consecutive reactions of anaerobic process: The multi-step nature of anaerobic biochemical process is illustrated in Figure 2.2 and can be summarized in the following steps.

(a) Hydrolysis: Large soluble organic molecules must be reduced in size to facilitate transport across the cell membrane. The reactions responsible for solubilization and size reduction are usually hydrolytic and are catalyzed by extra cellular enzymes produced by bacteria. Hydrolytic microorganisms will release exo-enzymes that break down large molecules like proteins, fats and starch (polymers) outside the bacteria cells into smaller soluble molecules like amino acids, fatty acids and sugar (monomers) suitable for use as a sources of energy and cell carbon.

(b) Acidogenesis: The acidogenesis involves the bacterial conversion of compounds resulting from hydrolysis phase into identifiable lower-molecular-mass intermediate compounds like volatile fatty acids (VFA), alcohols,

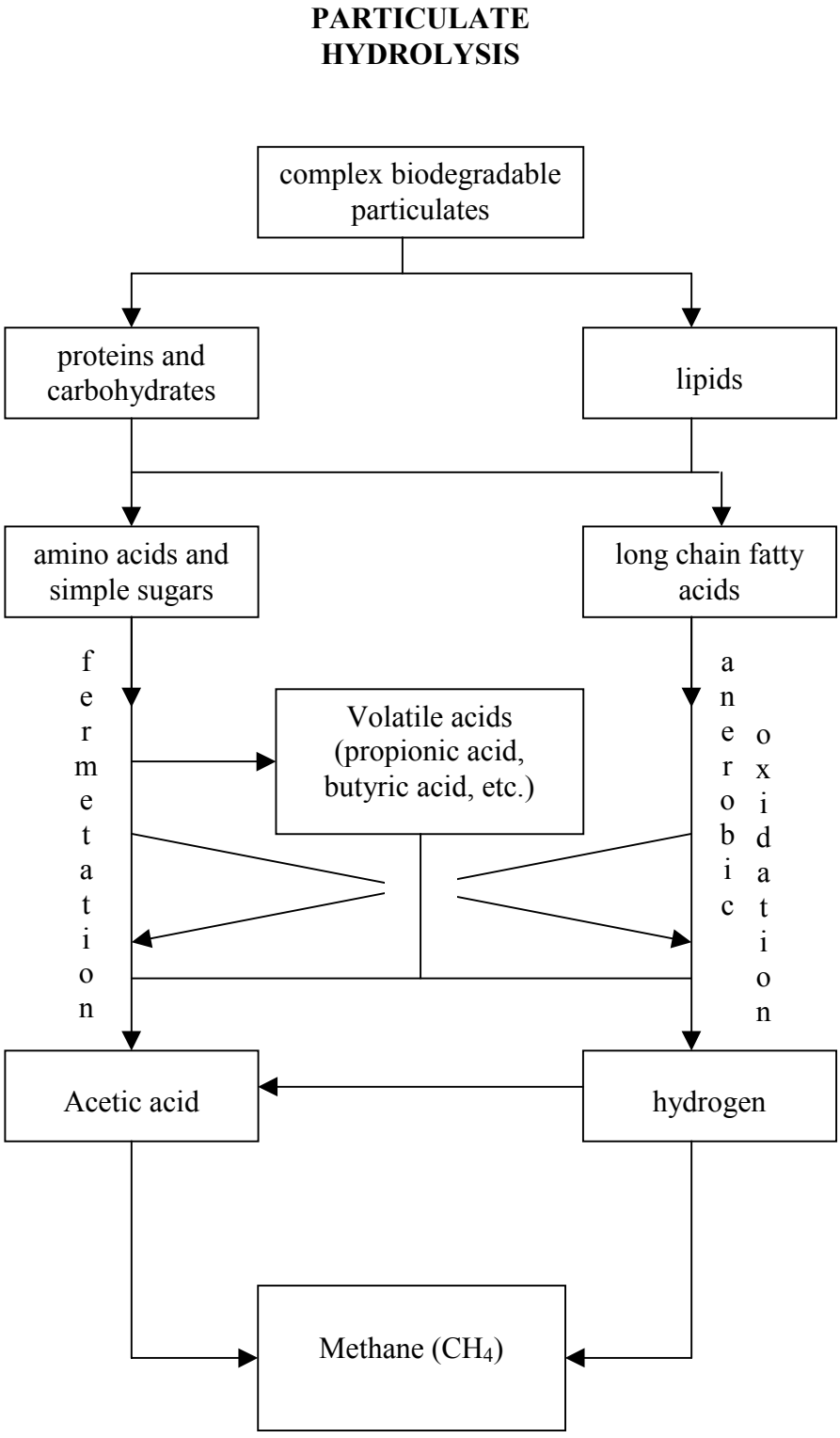



**Figure 2.2** Nature of anaerobic process

$CO_2$, and $H_2$. Main products of volatile fatty acids are formic acid, acetic acid, propionic acid and butyric acid and these acids are converted into acetic acid by acetogenic bacteria. The following chemical reaction is the example of acidification of glucose to acetic acid.

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \quad (2\text{-}1)$$

Furthermore, by-products like $H_2S$ and $NH_3$ may develop in this phase.

(c) Methanogenesis: The products of the acidogenic reactions, acetic acid and $H_2$ are used by methanogens, which are members of the domain Archaea, to produce methane gas. Two groups are involved (1) acetoclastic methanogens, which split acetic acid into methane and carbon dioxide as the following chemical reaction:

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (2\text{-}2)$$

and (2) $H_2$-oxidizing methanogens, which reduce carbon dioxide as the following chemical reaction.

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (2\text{-}3)$$

2) Anaerobic bacteria: The hydrolytic and fermentative bacteria comprise rather diverse groups of facultative and obligatory anaerobic bacteria. The most important hydrolytic and fermentative reactions are performed by strict anaerobes such as Bacteroides, Clostridia, and Bifidobacteria; however, the nature of the substrate will determine the species present.

The role of $H_2$ as an electron sink is central to the production of acetic acid as the major end product of acidogenesis. Reactions leading from long chain fatty acids, volatile acids, amino acids, and carbohydrates to acetic acid and $H_2$ are thermodynamically unfavorable under standard conditions, having positive

standard free energies (Grady et al., 1999). The bacteria which produce $H_2$ is obligatory linked to the methanogens that use it. Only when the methanogens continually remove $H_2$ by forming methane will the $H_2$ partial pressure be kept low enough to allow production of acetic acid and $H_2$ as the end products of acidogenesis. However, because $H_2$ partial pressures are kept low in anaerobic biochemical operations, $H_2$-forming bacteria play a little role. Several species have been identified, including members of the genus Syntrophomonas, which oxidize fatty acids, and the genus Syntrophobacter, which oxidize propionate.

The major nuisance organisms in anaerobic operations are the sulfate-reducing bacteria, which can be a problem when the wastewater contains significant concentrations of sulfate.

The previous studies of dairy wastewater treated by anaerobic biological treatment process are as follows (Panesar et al., 1999).

3) Anaerobic Lagoons: This is one of the oldest form of anaerobic digestion, requiring large space and have low capital and operation cost. A BOD reduction of about 90 % may be obtained with retention time of 7 days and organic loading rate of 4.48 kg BOD/$m^3$/d.

4) Anaerobic Filters: In anaerobic filters both stone media filters and PVC filters are used for treating dairy effluents. The COD loading ranging between 0.8 to 3.6 kg BOD/$m^3$/d are used and COD removal efficiencies of 91% to 82% respectively have been reported. The disadvantage of PVC filter plastic media is its high capital cost.

5) UASB reactor: The UASB reactors are becoming more popular for treatment of dairy effluents due to its lower operation and maintenance cost. The

hydraulic retention time of 12-18 h has been reported to give 85-90% BOD reduction and 75-80% COD reduction. The methane production of 0.25-0.31 $m^3$/kg COD reduced is reported with the biogas having 75-89% methane content. UASB process is successfully used at Vasudhara Dairy in Gujarat, India with a saving of about 50% in terms of power, as compared to conventional process. An additional advantage is that the UASB system treating 1000 $m^3$/d wastewater having COD of 2000 kg/d can produce approximately 830 $m^3$ of biogas.

## 2.4 Microbiology of Anaerobic Process

### 2.4.1 Types of Microbial in Anaerobic Process

As mentioned in Section 2.3.2, the biological conversion of the organic matter in anaerobic wastewater treatment process was found to occur in 3 steps. The first step in the process involves the enzyme-mediated transformation (hydrolysis) of higher-molecular-mass compounds into compounds suitable for use as a source of energy and cell carbon. The second step (acidogenesis) involves the bacterial conversion of the compounds resulting from the first step into identifiable lower-molecular-mass intermediate compounds. Anaerobic acidogenesis is known as the first step in the anaerobic digestion of soluble organic materials to methane and $CO_2$. Many kinds of bacteria are involved in the acidogenesis, subsequently, several kinds of organic acids and alcohol are usually produced (Horiuchi et al., 2002). The third step (methanogenesis) involves the bacterial conversion of the intermediate compounds into simple products, principally methane and carbon dioxide. In a reactor, anaerobic microorganisms work harmoniously to bring about the conversion of organic wastes.

One group of microorganisms is responsible for hydrolyzing organic

polymers to basic structural compound. A second group of anaerobic bacteria ferments the breakdown products to simple organic acids. This group of microorganisms, called as nonmethanogenic, consists of facultative and obligate anaerobic bacteria. Normally, these microorganisms are often determined as acidogens. Various kinds of the non-methanogens reviewed by Wajanavijai (1991) are illustrated in Table 2.3. A third group of microorganisms converts the hydrogen and acetate formed by the acid formers to methane gas and carbon dioxide. The bacteria responsible for this conversion are strict anaerobes, called methanogens or methane formers. Table 2.4 shows a number of revised grouping of the methanogens reported by Wajanavijai (1991).

Methanogens have been isolated from a wide range of anaerobic habitats where complex organic matter is deposited. The complex organics are firstly converted to a mixture of volatile fatty acids, alcohols, carbon dioxide, and $H_2$ by the fermentative group. The fatty acids and alcohols are converted to acetic acid at the expense of the reduction of protons to $H_2$ (Abbanat et al., 1989). The three orders of methanogens (Methanobacteriales, Methanococcales, and Methanomicrobiales) are characterized by extreme morphological diversity and the ability to proliferate in harsh environments including saturated brines and temperatures over $90^oC$. The characteristics of contemporary methanogens, and other archaeobacteria, has led to a hypothesis in which the ancestral organism was strictly anaerobic thermophile that originated when the earth temperature was high. Two pathways of methane formation are important in the dissimilation of complex organic matter: (i) the conversion of acetate to methane and carbon dioxide as shown in chemical reaction (2-2), and (ii) the reduction of carbon dioxide with $H_2$ or formate as the electron donors as illustrated

**Table 2.3** The non-methanogens that has been found in anaerobic digesters.

| Genus | Bacterial species |
|---|---|
| Aerobacter | A.aerogens |
| Aeromonas | Aeromonas sp. |
| Alcaligens | A.bookerii |
| | A.faccalis |
| | A.fiscolactis |
| | A.viscolactis |
| Bacillus | B.cereus |
| | B.cereus var mycoides |
| | B.circulans |
| | B.firmus |
| | B.knefelkampi |
| | B.megaterium |
| | B.pantothenticus |
| | B.pumilis |
| | B.sphaericus |
| | B.subtilis |
| | Bacillus sp. |
| Bacteroides | Bacteroides sp. |
| Clostridium | C.aminovalericum |
| | C.carmofoetidum |
| Escherichia | E.coli |
| | E.intermedia |
| | Escherichia sp. |
| Klebsielia | Klebsiella sp. |
| Leptospira | L.bitlexa |
| | Leptospira sp. |
| Micrococcus | M.candidus |
| | M.luteus |
| | M.carians |
| | M.urae |
| | Micrococcus sp. |
| Neisseria | N.catarrhalls |
| Paracolobacirun | P.intermedium |
| | P.coliforme |
| Proteus | P.vulgaris |
| Pseudonionas | P.acruginosa |
| | P.ambigua |
| | P.denitrificans |
| | P.oleovorans |
| | P.perolens |
| | P.pseudomallei |
| | P.reptilivora |
| | P.riboflavina |
| | Pseudonionas sp. |
| Rhodopscudomonas | R.pulusiris |
| Sarclna | S.coolsonli |
| | S.lutea |
| Serratia | S.indlcans |
| Streptococcus | S.diploidus |
| Streptomyces | S.bikiniesis |

**Table 2.4** A revised grouping of the methanogen classified by using the 165 ribosomal RNA sequences and substrates.

| Species | Former designation | Substrates for growth and $CH_4$ production |
|---|---|---|
| Order I. Methanobacteriales (type order) | | |
| Family I. Methanobacteriaceae | | |
| Genus I. Methanobacterium (type genus) | | |
| 1. Methanobacterium formicicum (neotype species) | Methanobacterium formicicum | $H_2$, formate |
| 2. Methanobacterium bryantii | Methanobacterium sp. strain M.o.H. | $H_2$ |
| Methanobacteriumbryantii strain M.o.H.G. | Methanobacterium sp. strain M.o.H.G. | $H_2$ |
| 3. Methanobacterium thermoautotrophicum | Methanobacterium thermoautotrophicum | $H_2$ |
| Genus II. Methanobrevibacter | | |
| 1. Methanobrevibacter ruminantium (type species) | Methanobacterium ruminantium strain MI | $H_2$, formate |
| 2. Methanobrevibacter arboriphilus | 1 Methanobacterium arbophilicum | $H_2$ |
| Methanobrevibacter arboriphilus strain AZ | Methanobacterium sp. strain AZ | $H_2$ |
| Methanobrevibacter arboriphilus strain DC | Methanobacterium strain DC | $H_2$ |
| 3. Methanobrevibacter smithii | Methanobacterium ruminantium strain PS | $H_2$, formate |
| Order II. Methanococcales | | |
| Family I. Methanococceae | | |
| Genus I. Methanococcus | | |
| 1. Methanococcus vannielii (neotype species) | Methanococcus vannielii | $H_2$, formate |
| 2. Methanococcus voltae | Methanococcus sp. strain PS | $H_2$, formate |
| Order III. Methanomicrobiales | | |
| Family I. Methanomicrobiaceae (type family) | | |
| Genus I. Methanomicrobium (type genus) | | |
| 1. Methanomicrobium mobile (type species) | Methanobacterium mobile | $H_2$, formate |
| Genus II. Methanogenium | | |
| 1. Methanogenium cariaci (type species) | Cariaco isolate JR1 | $H_2$, formate |
| 2. Methanogenium marisnigri | Black Sea isolate JR1 | $H_2$, formate |
| Genus III. Methanospirillum | | |
| 1.Methanospirillum hungatii | 2 Methanospisillum hungatii | $H_2$, formate |
| Family II. Methanosarcinaceae | | |
| Genus II. Methanosarcina (type genus) | 3 Methanosarcina barkeri | $H_2$, $CH_3OH$, $CH_3NH_2$, acetate |
| 1. Methanosarcina barkeri (type species) | Methanosarcina barker strain 227 | $H_2$, $CH_3OH$, $CH_3NH_2$, acetate |
| 2. Methanosarcina barkeri strain 227 | Methanosarcina barkeri strain W | $H_2$, $CH_3OH$, $CH_3NH_2$, acetate |
| 3. Methanosarcina barkeri strain W | | |

Source : Wajanavijai (1991)

in chemical reaction (2-3). Sawyer et al. (1994) reported that about 70 of the methane resulting from the complete methane fermentation of complex wasted results from fermentation of acetic acid. This step is a very slow process, which is the rate-limiting step of anaerobic digestion. Methane formers are generally known as very sensitive to disturbances. Regarding to $CO_2$ production, change in $CO_2$ content can forecast difficulties in anaerobic digestion process. In normal digestion, $CO_2$ is released due to decomposition of the organic matter, a combination of it and ammonia, also produced by biological activity, provides a source of alkalinity. As the process becomes retarded and volatile acids begin to accumulate, the acids tend to react with the available alkalinity to form salts of the acids and release $CO_2$ from the system, as illustrated in chemical reaction (2-4) below.

$$R\text{-}COOH + NH_4HCO_3 \rightarrow R\text{-}COO.NH_4 + CO_2 + H_2O \quad (2\text{-}4)$$

Therefore, because the methane-producing mechanism is inhibited during the accumulation of volatile acids, the percentage of $CO_2$ released is increased. This is accompanied by a decrease in $CH_4$ content of the digester gas.

Sulfate-reducing bacteria (SRB) is one type of anaerobes that use sulfate ions as the terminal electron acceptor for the metabolism of organic substrates. Sulfate reduction is carried out by SRB as shown in the following equations, whereas $CH_2O$ represents a carbohydrate.

$$2CH_2O + SO_4^{2-} \rightarrow S^{2-} + 2CO_2 + 2H_2O \quad (2\text{-}5)$$

$$S^{2-} + 2CO_2 + 2H_2O \rightarrow H_2S + 2HCO_3^- \quad (2\text{-}6)$$

It indicated that SRB could be used to remediate the pollution problem of acid mine drainage by converting sulfate to sulfite that could be compelled out to the atmosphere as $H_2S$ gas (Elliott et al., 1998).

Jetten et al. (2001) studied the microbiology and application of the anaerobic ammonium oxidation process and found that anammox process was catalyzed by a group of deep-branching planctomycetes, including Candiadatus B. anammoxidans and Candidatus K. stuttgartiensis. Co-cultures of oxic and anoxic ammonia-oxidizing bacteria convert ammonia directly to dinitrogen gas under oxygen limitation. Furthermore, this study indicated that K. stuttgartiensis was in many ways similar to B. anammoxidans.

Investigation of anaerobic metabolism of Bacillus subtilis reported by Ye and Thomas (2001) indicated that Bacillus subtilis was traditionally believed to be a strict aerobe. It turns out that Bacillus subtilis can carry out anaerobic dissimilatory reduction of nitrate to ammonia via nitrite. This anaerobic process has long been considered to be a way of dissipating electrons under anaerobic conditions. However Bacillus subtilis is capable of using nitrate and nitrite as the alternative electron acceptors to support anaerobic growth.

Holliger (1995) studied the anaerobic microbiology and biotreatment of chlorinated ethenes. This study result indicated that the reductive dechlorination of perchloroethylene (PCE) and trichloroethene (TCE) to ethene observed in aquifers under natural conditions, anaerobic bioreactors, and in enrichment cultures. Most of them were mediated by bacteria that utilized the chlorinated ethenes as electron acceptors in a respiratory process, rather than by methanogens and acetogens that metabolically dechlorinate PCE to TCE.

Moreover, numerous studies have shown that anaerobic bacteria are capable of reductive dehalogenation during biotransformation of TCE (Distefano, 1999).

### 2.4.2 Types of Granulation Sludge

Referring to granulation sludge, the different types of granulation sludge may develop on the nature of the seed sludge, the composition of substrate, and the conditions applied during the start up. The followings are the types of granulation sludge (Wajanawijai, 1991).

1) Sarcina granules developed when a high concentration of acetic acid was maintained in the reactor, i.e., methanosarcnia with diameter of approximately 0.5 mm.

2) Rod granules consisted predominantly of rod shaped bacteria in fragments of approximately five cells. It developed not only on the potato-processing waste and sugar-beet wastes in full scale plants, but also on VFA substrate when the digested sewage had been enriched with a small amount of granular sludge of the "rod" type, i.e., methanothrix with diameter of approximately 2 mm.

3) Filamentous granules mainly consisted of long multicellular rod-shaped bacteria. These granules developed on pure VFA substrate and digested sewage sludge of a relatively high specific methanogenic activity (0.12 $KgCH_4$-COD/ Kg VSS-d), i.e., Methanothrix soehngenii with diameter of approximately 5 mm.

4) Spilky granules were very uniform in shape and size, contained up to 60% $CaCO_3$. These granules were up to 1 mm long and less than 0.5 mm thick, and developed on maize-starch waste in a 900 $m^3$ full scale UASB.

### 2.4.3 Granular Formation

For bacteria in an anaerobic culture to form granules, Liu et al. (2003) summarized the mechanisms for the formation of anaerobic granules that can be classified into 4-step model as follows.

Step 1: Physical movement to initiate bacterium-to-bacterium contact or bacterial attachment onto nuclei. The forces involved in this step are: hydrodynamic force, diffusion force, gravity force, thermodynamic forces, e.g. Brownian movement, and cell mobility. Cells can move by means of flagella, cilia and pseudopods, while cell movement may also be directed by a signaling mechanism.

Step 2: Initial attractive forces to keep stable multicellular contacts. Those attractive forces are:

Physical forces: Van de Waals forces, opposite charge attraction, Thermodynamic forces including free energy of surface, surface tension, hydrophobicity, and filamentous bacteria that can serve bridge to link or grasp individual cells together.

Chemical forces: hydrogen liaison, formation of ionic pairs, formation of ionic triplet, and interparticulate bridge and so on.

Biochemicals forces: cellular surface dehydration, cellular membrane fusion, and signaling and collective action in bacterial community.

Step 3: Microbial forces to make cell aggregation mature: production of extracellular polymer by bacteria, such as exopolysaccharides etc, growth of cellular cluster, and metabolic change and genetic competence induced by environment, which facilitate the cell-cell interaction, and results in a highly organized microbial structure.

Step 4: Steady state three-dimensional structure of microbial aggregate shaped by hydrodynamic shear forces. The microbial aggregated would be finally shaped by hydrodynamic shear force to form a certain structured community. The outer shape and size of microbial aggregates are determined by the interactive strength/pattern between aggregated and of hydrodynamic shear force, microbial

species and substrate loading rate.

### 2.4.4 Influence Factors on Granule Formation

Regarding to the granular formation, the followings are the influence factors.

1) Types of the seed sludge: Lettinga et al. (1985) reported that two types of digested sewage sludge as proper seed materials: thicker types as approximately 60 kilogram dry solids/$m^3$ (Kg DS/ $m^3$) and thinner types (<40 Kg DS/ $m^3$). The thicker types are preferred because of better settleability.

2) Types of wastewater: According to the obtained result in mesophilic UASB reactor carried out by Lettinga et al. (1985), the granulation was found to proceed faster for lower strength waste. They pointed out that the heavier fraction would be retained in the reactor, while the finely dispersed matter was washed out. Therefore, they recommended to apply the effluent recycle system in case that the UASB-reactor must be started up for higher strength wastes such as COD concentration exceeded 5,000 mg/L. Switzenbaum (1983) indicated that development of the pelletized sludge depended on the characteristics of the wastewater an inoculum used when starting the reactor.

3) Physical and chemical condition: pH value and alkalinity: the proper pH value in reactor should be kept in the ranges of 6.5-7.8, however, some researcher suggested that the difference was not observed for the pH ranges of 6.3-8.1 (Wajanawijai, 1991).

- Temperature: The mesophilic temperature of 35-40$^oC$ was mentioned as the optimum condition, based on the activities as well as other operation aspects (Wajanawijai, 1991).

- Effect of $Ca^{2+}$: Various related studies were reported that the washout of sludge during the initial step of start-up could be reduced by increasing the $Ca^{2+}$ concentration in the feed solution. Forster (1991) reported that the stimulation of granulation by feed $Ca^{2+}$ concentration up to 100 mg/L, while Alibhai and Forster (1986) found that both calcium, at 80 mg/L, and phosphate, at 192 mg/L, improved granulation when added either one. Whereas granules of greater stability were produced when added together. Switzenbaum (1983) reported that several factors influence the development and characteristics of these particles, including nutrient supply, organic loading rate and calcium concentration.

- Effect of $NH_4^+$: A concentration of ammonia is often considered as an important factor affecting the process performance. The adverse effect of ammonia is found when $NH_4^+$-concentrations are as high as 1,000 mg/L. Grady et al. (1999) indicated that ammonia was inhibitory in anaerobic process at higher concentration or greater than 200 mg/L as N and toxic if the concentration is high enough. Ammonia may be present in the influent wastewater, or it may be formed as a result of the breakdown of organic materials that contain nitrogen. Ammonia is a weak base and dissociates in water as indicated in the following reaction.

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^- \qquad (2\text{-}7)$$

Those species ($NH_3$ and $NH_4^+$) are inhibitory, but at significantly different concentrations. Free ammonia ($NH_3$) is more inhibitory and can cause a toxic response at concentrations of about 100 mg/L as N (Parkin and Owen, 1986). On the other hand, ammonium ion ($NH_4^+$) concentrations as low as 1,500 mg/L as N have been reported to be toxic (McCarty, 1964).

- Inhibitors and toxic compounds: these substances, including

formaldehyde, cyanide, and etc., should be absent in the feed, if possible, especially during the initial stage of granulation, because they can kill the bacterial population in the digester (Lettinga et al., 1985). Other researchers suggested that iron might be a limiting factor in the growth of thermophilic bacterium Methanobacterium thermoautotropicum (Wajanawijai, 1991).

4) Nutrients: The growth parameters of macro nutrients, i.e., N, P and S must be present with sufficient concentrations in available form. The requirement of COD: N: P for the cultivation of granular sludge is the same as that in general anaerobic process. Lettinga et al. (1985) revealed that an application for S, the experimental results obtained in batch fed experiment with VFA-feeds, had positive effect on growth of methanogenic bacteria upon addition of 0.1 mM/L $S^{2-}$.

In case of inorganic nitrogen compound, the metabolism of them plays many important physiological roles in microorganisms. Denitrification, a process of converting nitrate to nitrous oxide or dinitrogen gas, allows microbes to use alternative electron acceptors to gain energy under oxygen-limiting condition. Nitrite can be reduced to nitric oxide under oxygen-limiting conditions. Dissimilatory reduction of nitrate to ammonia via nitrite can support anaerobic growth of some bacteria. Ammonia oxidizers oxidize ammonia to hydroxylamine, that was subsequently converted to nitrite. The nitrite produced could be converted to nitrate by nitrite oxidizers. Ammonia could be oxidized by two pathways: firstly, ammonia was oxidized to hydroxylamine oxidoreductase as illustrated in Figure 2.3; and secondly, ammonia and nitrite were anaerobically converted to dinitrogen gas as shown in Figure 2.4 (Ye and Thomas, 2001). Anaerobic ammonium Ammonia and hydroxylamine are converted to hydrazine by a membrane-bound enzyme. Hydrazine

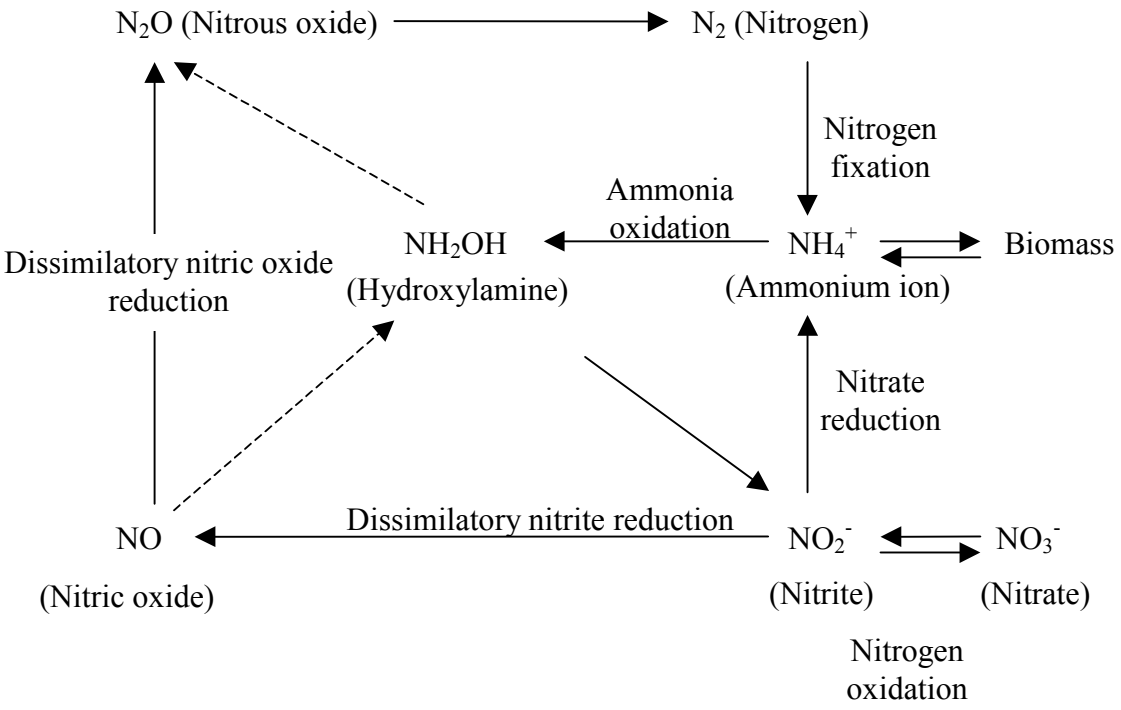



**Figure 2.3** Nitrogen cycle in anaerobic process.

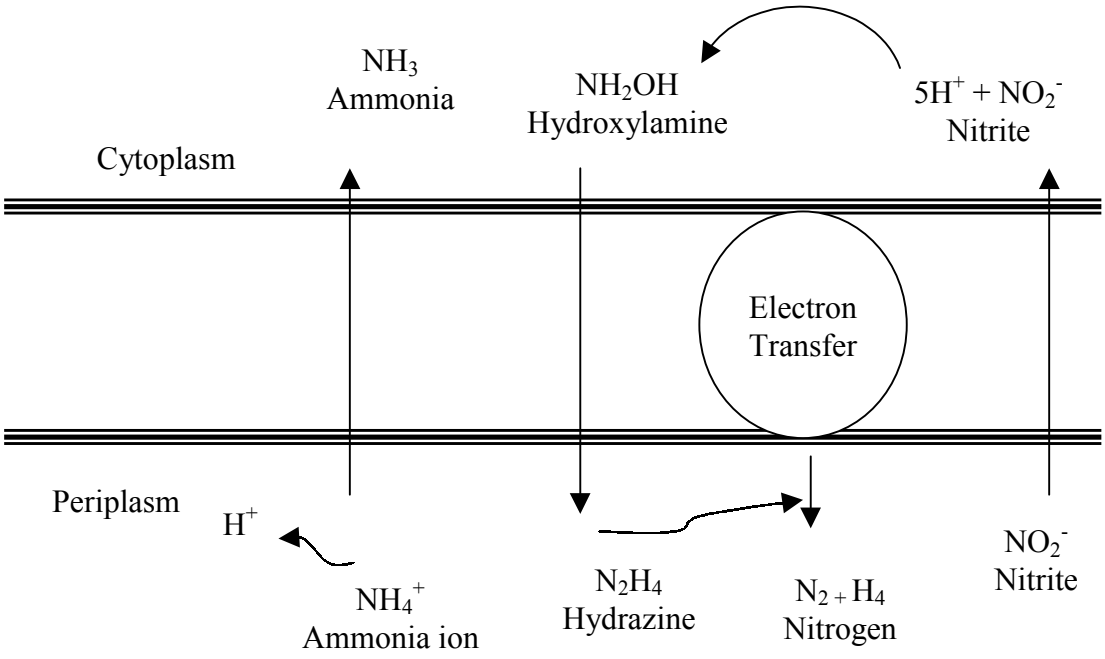



**Figure 2.4** Anaerobic ammonium oxidation (anammox) by Planctomycetes.

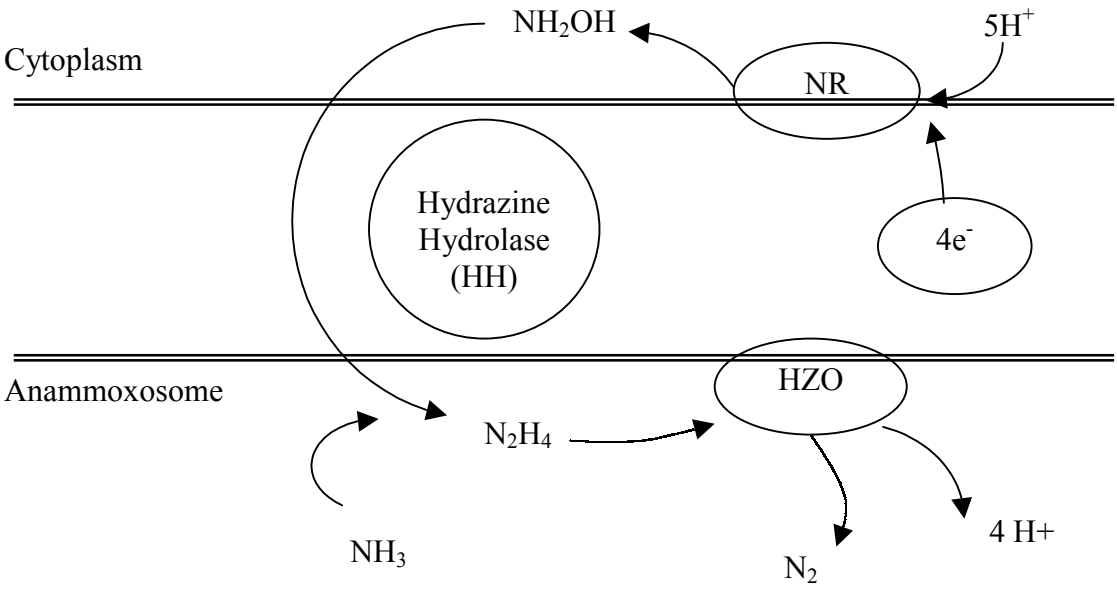
is oxidized in the periplasm. Ye and Thomas (2001) proposed two systems describing the mechanism of electron transfer for nitrite reduction: one system involves a single enzyme that is responsible for hydrazine oxidation and nitrite reduction, and the other involves a nitrite-reducing enzyme that mediates formation of hydroxylamine while an electron transport chain enzyme supplies the electrons.

Jetten et al. (2001) also investigated the possible metabolic pathway for anaerobic ammonium oxidation using N-labeling experiments. The obtained results indicated that the electron acceptor nitrite was reduced to hydroxylamine and that hydroxylamine somehow reacted with the electron donor ammonium, leading to the ultimate production of dinitrogen gas. In batch experiments with excess hydraoxylamine and ammonium, a transient accumulation of hydrazine was observed.

The oxidation of hydrazine to dinitrogen gas generated the electrons for the initial reduction of nitrite to hydroxylamine as illustrated in Figure 2.5.

Concerning with phosphorus constituent, milk is a very rich source of phosphorus (Wendorff, 1997), whole milk contains an average of 93 mg of phosphorus per 100 g of milk, then the dairy wastewater must consist of phosphorus. Biological phosphorus removal is a complex process that is dependent on the growth of specialized Phosphate Accumulating Organisms (PAOs), which store phosphorus as polyphosphate (Poly-P) as shown in Figure 2.6. Under anaerobic conditions, PAOs do not grow, but store acetic acid as PHB (Poly-hydroxybutyrate) in endogenous respiration that may affect the formation of methane.

5) Operational factors: The most significant operation factors are stable organic loading and higher hydraulic loading rate (Wajanavijai, 1991).




HH : hydrazine hydrolase

NR : nitrite-reducing enzyme

HZO : hydrazine – oxidizing enzyme

**Figure 2.5** Mechanism of anaerobic ammonium oxidation.

Lettinga et al. (1985) indicated that as for the higher hydraulic loading rate, the lighter sludge would move upward easily whereas the heavier sludge would move downward. It was reported by Liu et al. (2003) that a simple and practical way towards the rapid anaerobic granulation was to increase the organic loading rate based on an 80% reduction of biodegradable COD with supplementary monitoring of effluent suspended solid washout.

### 2.4.5 Microbiology in UASB Process

Forster (1991) studied anaerobic upflow sludge blanket reactor: aspects of their microbiology and their chemistry obtained from Dutch digesters, indicated that Methanothrix was found to be present in all the stable granules that were

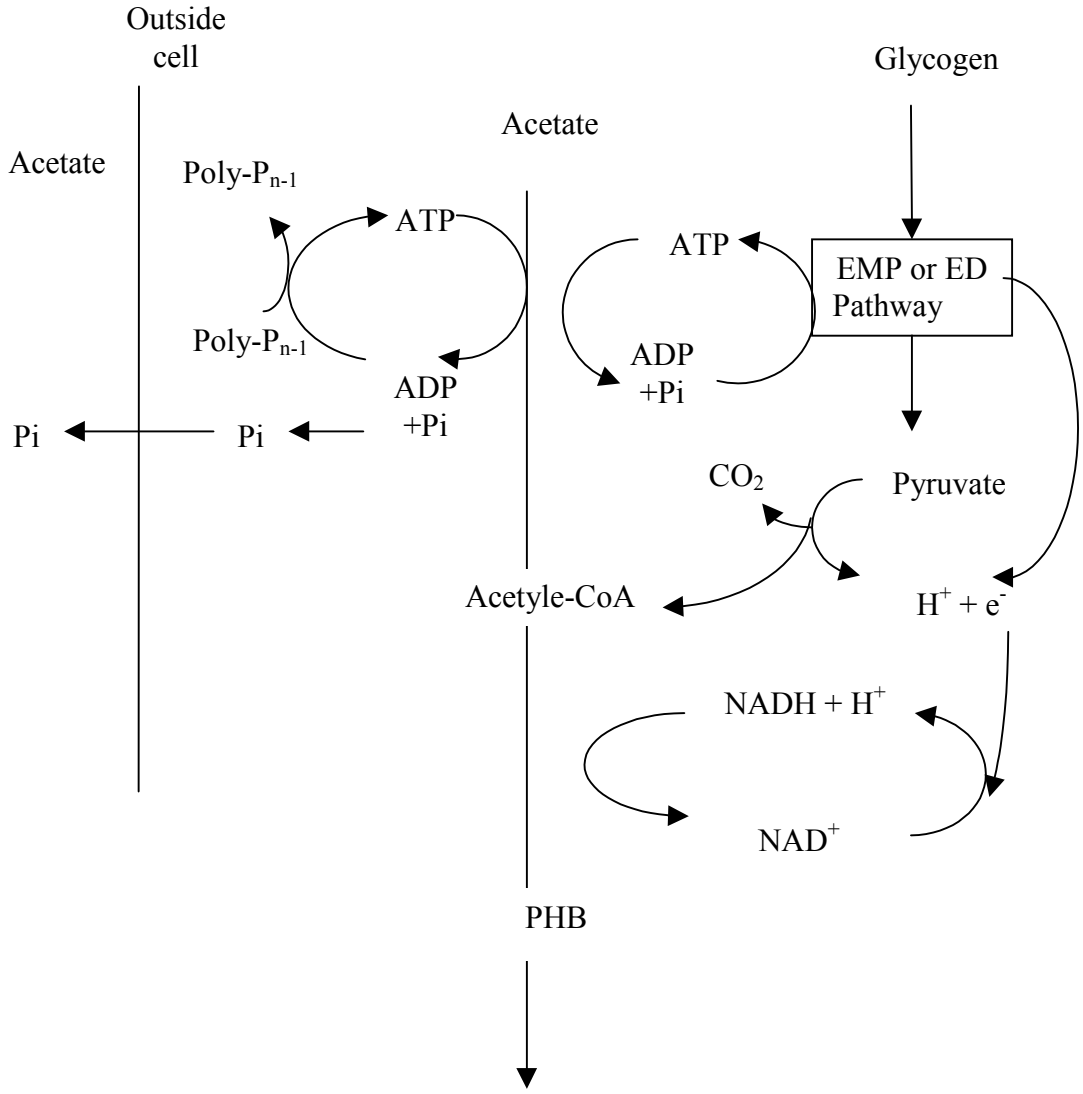



**Figure 2.6** Mino model for the uptake and release of inorganic phosphorus by PAOs.

examined. Whereas in loosely aggregated non-granular sludge the dominant species was a thin filamentous strain.

Wajanawijai (1991) also revealed the importance of Methanothrix in determining the morphological development of the biomass in UASB reactor. In addition to Methanothrix, Methanosarcina and Methanobacterium formicium were

dominant in granular UASB sludge. The ecology of the biomass in anaerobic reactors is not only important in determining the initiation and the stability of the immobilization process, but is also a significant factor in defining the rate at which acidogenesis and methanogenesis take place.

McHugh et al. (2003) examined the methanogenic population structure in a variety of anaerobic bioreactors in six anaerobic sludge using culture-independent techniques and found that five separate groups of methanogens were represented with Methanosaeta-like species dominant in all sludge. Syutsubo et al. (1998) studied the granulation and sludge retainment during start-up of a thermophilic UASB reactor. They found that the optimum temperatures of methanogenic activities of 55$^{o}$C-cultivated sludge varied by tropic group. Both acetate- and hydrogen-utilizing methanogenic activities exhibited their optima at 65$^{o}$C, while that of propionate-fed methanogenic activity occurred at 50$^{o}$C. The propionate degradation was most likely to be the rate-limiting step in thermophilic anaerobic processes.

The granule found in the degradation of phenol in wastewater by using UASB reactor composted of, among others, Syntrophus buswelli-, Methanothrix-, Methanospisillum- and Methanobrevibacter-like bacteria (Fang et al. 1996). Similar to the study undertaken by the same researcher on the degradation of butyrate in a UASB digester, butyrate-degrading granules mainly composted of Methanotrix-like bacteria (Fang et al. 1995). The granules were 1-2 mm in size and had a densely-packed skin layer which comprised two types of microcolony: one was composted of cocci with abundant extracellular polymer and the other was composted of two bacterial species in juxtapositioned syntrophic association. Granulation of methanogenic bacteria in UASB reactor is important in the treatment of various

industrial wastewater containing toxic substances due to the compact structure that protects the bacteria from inhibitory and toxic pollutants.

## 2.5 Upflow Anaerobic Sludge Blanket Process

This reactor had been developed by Lettinga ever since 1971 (Wajanavijai, 1991). The basic idea underlying the concept is that a high sludge concentration can be maintained by mounting the settler in the upper part of the upflow reactor. Lettinga, who has improved a more effective settler, called it the Upflow Anaerobic Sludge Blanket (UASB) process. The basic operation of UASB is the feeding wastewater into the reactor from the bottom of bioreactor and leaves it at the top settler for separation of gas, sludge, and liquid. Then, the particles of sludge are settled back towards the digesting zone.

The key to successful operation of the UASB is to keep the sludge within the system (i.e. maintaining the solids without any support material). This accomplished with the internal Gas-Solids Separator (GSS) and by minimization of mechanical mixing and/or sludge recirculation for the sake of improving the ability of sludge to settle. The GSS located in the upper part of the reactor is particularly important. This separator allows the separation of entrapped or attached gas bubbles from the sludge and the return of sludge particles from the quiescent settler compartment to the digester compartment. One of the features of the UASB process is a so-called granular sludge in the reactor. The anaerobic sludge can be flocculated and formed into granules, which have excellent properties under proper physical and chemical conditions. A dense slurry of granules forms in the lower portion of the bio-reactor, and the combined effects of the influent wastewater distribution and gas production

result in the mixing of the influent wastewater with the granules. Treatment occurs within the dense blanket of granules. An important additional factor in the development of sludge of desired quality is the creation and maintenance of favorable conditions for flocculation within the system, i.e., the presence of $Ca^{2+}$ ions, adequate mixing, and the absence of a high concentration of poorly flocculating suspended matter in the wastewater. Moreover, sufficient nutrients should be present and available to ensure bacterial growth (Lettinga et al., 1980). In terms of design parameters, hydraulic retention times are in the range of 4 to 24-h for the UASB process.

Bioreactor dimensions are affected by process loading, constraints on maximum upflow velocities, wastewater type, and the settling characteristics of the solids that develop in the process. Bioreactor HRTs in the 0.2 to 2 day range are typical, along with volumetric organic loading (VOL) rates of 2 to 25 kg COD/$m^3$-d, depending on wastewater characteristics and whether granular or flocculent solids develop.

### 2.5.1 Past Studies on UASB Experiments with Various Wastewaters.

The first pilot experiments were carried out in 1976 with sugar-beet wastes. A large number and variety of wastewater have been investigated on both the laboratory and pilot-scale using UASB process. The relevant results of laboratory experiment by means of UASB are presented in Table 2.5 (Lettinga et al., 1980). In addition, the application of both full-scale and laboratory or pilot-scale systems can be categorized as municipal (low strength) applications and industrial (high strength) applications as follows.

1) Municipal applications: Lettinga et al. (1983) pointed the

**Table 2.5** Details Summary of some laboratory-scale UASB experiments with various types of wastes.

| Types of Waste | COD (mg/L) | HRT (h) | COD Removal (%) | Temperature ($^{o}$C) | Reactor Volume (L) | Height (cm) |
|---|---|---|---|---|---|---|
| Sugar-beet sap, unsoured | 5000-6000 | 48-24 | 95 | 30 | 61 | 105 |
| Sugar-beet sap, soured (closed circuit) | 6000-9500 | 12-24 | 84-95 | 30 | 18 | 70 |
| Sugar-beet sap, soured (2 stage) | 6000-9000 | 24 | 90-97 | 30 | 18 | 70 |
| Bean blanching | 5200 | 13-15 | 90-95 | 30 | 2.7 | 30 |
| Sauerkraut | 10000-20000 | 24 | 90-97 | 30 | 2.7 | 30 |
| Dairy | 1500 | 5 | 90 | 30 | 18 | 70 |

Source: Lettinga et al. (1980)

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attraction of the UASB process for the treatment of low strength wastes particularly for developing countries in tropical areas. Using a 120 liters 2 m high UASB pilot reactor, operated at 12-h HRT, raw domestic sewage was treated at temperatures as low as 8-20$^{o}$C with COD removal efficiencies of 65-85%. In case of heavy rainfall, the COD reduction dropped to 50-70%. Barbosa et al. (1989) used UASB treating raw domestic sewage, it was observed that the removal efficiency of $BOD_5$ and COD were 78 and 74%, respectively. Similar work carried out by Karnchanawong and Ninprayoon (1993) found that the removal efficiency of $BOD_5$ and COD were in the ranges of 76.9-92.9% and 76.4-88.1%, respectively. Kalogo et al. (2001) investigated the physical and biological performance of self-inoculated UASB reactor treating raw domestic sewage. After 22 weeks of operation at 29$^{o}$C with an HRT of 4 h, the reactor removed up to 80% of total COD, 60% of soluble COD, and 90% of SS. The results indicated that the operation of a UASB reactor with raw domestic sewage without inoculation was feasible.

2) Industrial applications: Numerous pilot-scale studies of the UASB process indicated successful treatment of industrial wastes with suggested design organic loading rates of 10-15 kg COD/$m^3$-day at 30$^{o}$C, with COD removals of the order of 75-80% (Switzenbaum, 1983).

The treatment of tapioca starch wastewater using UASB has been reported to achieve 95% COD reduction with gas productivity of 5-8 $m^3/m^3$.day (Annachhatre and Amatya, 2000). Using UASB for treating wastewater of rice powder plant was reported by Lakhanaadisorn et. al. (2002), to achieve 80-90% BOD reduction, while 90-95% COD reduction was recorded. Methane formation was 0.45 $m^3$ per kg of COD removal per day.

The brewery wastewater treatment was also studied using UASB by Yan and Tay (1996). The removal efficiency of COD and BOD were 89.1% and 91.3%, respectively, under the volumetric loading of 12.2 g COD/L-d. and hydraulic retention time of 4 h. Whereas Wannavijai (1991) reported the treatment efficiency of brewery wastewater using UASB reactor to be 90-94% and 94-97% for COD and BOD removal, respectively. Methane production was in the range of 188-316 L/kg COD removed and its content varied between 74.0 – 80.9 % of biogas produced.

Treatment of dairy wastewater using an upflow anaerobic sludge blanket reactor was also studied by Gavala et al. (1999). The performance of reactor was assessed by monitoring pH, COD, biogas production and composition. Operation at an organic loading rate of 6.2 g COD/L-d was found to be safe and could be increased to a maximum of 7.5 g COD/L-d. They recommended that a longer HRT would be required for treatment of non-diluted wastewater.

Rodriguez-Matinez et al. (2002) used UASB for treating slaughterhouse wastewater, 88.8% of removal efficiency was achieved. Removal efficiencies for phosphate, total suspended solids, nitrogen and nitrates were 39, 90.3, 71.8 and 78.1%, respectively.

The UASB process could be used to treat not only the nutrient contained in the wastewater but also the other chemicals. Fang et al. (1996) studied the degradation of phenol in wastewater in an UASB reactor and indicated that over 97% of phenol was removed at 37$^{o}$C and pH ranging 6.9-7.5 with 12-h of hydraulic retention time for phenol concentration up to 1,260 mg/L. The study on the degradation of butyrate in an UASB reactor was also undertaken by the same researchers and they found that the conversion of acetate to methane appeared to be the rate-limiting step (Fang et al.,

1995b). Of all the COD removed, 94.5% was converted to methane; the average sludge yield was 0-0.37 g VSS/g COD. The same researchers (Fang et al., 1995a) also used UASB for treating propionate-rich wastewater; COD removal was monitored to be as high as 97-99%. The percentage of COD conversion to methane was similar to the degradation of butyrate with 95% and the rest was converted to biomass with the average yield of 0.040 g-VSS/ g-COD.

Rinzenma (1993) studied anaerobic digestion of long-chain fatty acids in UASB reactors and found that the process was unsuitable if lipids contributed 50% or more to the COD of wastewater as the gas production rate required to obtain sufficient mixing and contact could not be achieved. At lipid loading rates exceeding 2-3 kg COD/$m^3$-d, total sludge washout occurred. At lower loading rates, the system was unreliable due to unpredictable sludge flotation.

## 2.6 Factors Influencing the Operation of UASB Process

### 2.6.1 Cell Residence Time

Cell residence time controls the types of microorganisms that can grow in the process and the extent to which various reactions will occur. Determination of the cell residence time is straightforward in flow-through systems such as anaerobic digesters, where it simply equals the HRT. In the various experiments using UASB reactors, very short HRT (3-4 h) could be applied with low and medium concentration of wastewater (1-3 kg/$m^3$ of COD). The HRT could be increased upto 1 day for the increased COD concentration (10-50 kg/$m^3$ of COD). Barbosa et al. (1989) evaluated the performance of UASB reactor with the HRT of 4 h, COD removal efficiency was 74%. Some researchers (Wu and Hickey, 1997) suggested that the hydraulic loading

rate in the range of 0.25-0.4 $m^3/m^2$-h was high enough for granulation. Karnchanawong and Ninprayoon (1993) recommended that the treatment efficiencies of UASB process for HRT in the range 12-24 h were not significantly different. At HRT lower than 9 h, the percent removal of organic matter decreased with decreasing HRT. Gavala et al. (1999) suggested that high retention times are required for non-diluted wastewater (COD of 60 g/L). Seghezzo et al. (1998) mentioned that the success of UASB reactors was mainly dependent on the sludge retention time (SRT), which was the key factor in determining the ultimate amount of hydrolysis and methanogenesis in UASB system at certain temperature conditions. Yan and Tay (1996) studied brewery wastewater treatment in UASB reactor at ambient temperature and found that the strategy of raising volumetric loading rate (VLR) and sludge loading rate (SLR) was based on COD removal efficiency of about 80% through corresponding stepwise reduction of hydraulic retention time (HRT). When HRT was reduced to 6 h, the sludge blanket was expanding and the sludge was lost excessively with 635 mg SS/L in the effluent on 3 consecutive days. Wajanawijai (1991) also indicated that the upflow velocity increased with lower HRT, causing more solids loss from the system.

### 2.6.2 Temperature

The performance of anaerobic processes is significantly affected by operating temperature. The results of the UASB experiments, applied for various types of wastewater, indicated that the temperature has a crucial effect on the system. Most of the highly-efficient treatment (the removal efficiency of COD > 90%) was obtained from the system operated under mesophilic temperature. Yan and Tay (1996) stated that temperature is a crucial factor that affects granules. Granulation is

achieved successfully at lower temperature. Fang et al. (1996) indicated that temperature change from 37 to 20$^{o}$C had only mild effect on the performance of reactors; all of them recovered fully within 2 days when the temperature returned to 37$^{o}$C. The only UASB reactor used in their study for treating phenolic wastewater exhibited high sensitivity to the temperature shock. Lettinga et al. (1983) suggested guidelines for designing the capacity of UASB reactors treating mainly soluble wastes, related to temperature as shown in Table 2.6.

**Table 2.6** Tentative design loading rates for UASB reactors in relation to the temperature.

| Temperature($^{o}$C) | Design loading rates (kgCOD/m$^{3}$-d) |
|---|---|
| 40 | 15-25 |
| 30 | 10-15 |
| 20 | 5-10 |
| 15 | 2-5 |
| 10 | 1-3 |

Source: Lettinga et al. (1983)

### 2.6.3 pH

pH has a significant impact on the performance of anaerobic process, with activity decreasing as the pH deviates from an optimum value. This effect is particularly significant for anaerobic processes because the methanogens are affected to a greater extent than are other microorganisms in the microbial community. A pH range of 6.8 to 7.4 generally provides optimum conditions for the methanogens,

whereas a pH between 6.4 and 7.8 is considered necessary to maintain adequate activity. pH will also affect the activity of the acidogenic bacteria; however, the effect is less significant and primarily influences the nature of their products. Horiuchi et al., (2002) reported the following effects of pH shift on the anaerobic acidogenesis: (i) the main products changed from butyric acid to acetic and propionic acids, depending on the culture pH shift from 5.0 to 8.0; (ii) the phenomenon was reproducible and reversible, and was not affected by the dilution rate; and (iii) pH control was effective for selective production of various organic acids from organic wastes.

Yu and Fang (2002) concluded that the degradation of dairy wastewater pollutants increased with pH shift from 4.0 to 5.5. At pH 5.5, 95% of carbohydrates, 82% of proteins and 41% of lipids were degraded. Further increase of pH, up to 6.5, increased degradation of carbohydrates, proteins and lipids only slightly, but resulted in the lowering of overall acid and alcohol production due to their increased conversion into methane.

Kalogo (2001) reported that during the second phase of UASB operation (day 55 to 94), the pH of the effluent constantly dropped, indicating that acid-producing metabolic reactions were occurring. There was however no excessive acidification in the reactor because the pH never dropped below 6.8.

The advantages and disadvantages of UASB can be summarized in Table 2.7.

## 2.7 Kinetics of Biomass Growth

Several mathematical models to characterize the anaerobic digester process have been developed. Among them, the Monod model has been mostly used. This

**Table 2.7** Advantages and disadvantages of UASB process.

| Advantages | Disadvantages |
|---|---|
| 1. A cheap treatment process.<br>2. UASB process uses no packing material to support sludge.<br>3. It requires low energy input.<br>4. Methane can be obtained and can be used as substitute energy.<br>5. UASB process has low excess sludge yield which eases disposal problems.<br>6. It requires limited area.<br>7. A high concentration of an active granular sludge retained in the reactor is capable to handle high organic loading and fluctuating wastewater characteristics, which may be changed from production process or any accidental spillage.<br>8. Granular sludge remained is viable in the reactor for a long period without additional feeding.<br>9. High quality effluent is achievable. | 1. Performance is dependent on development of dense settleable solids.<br>2. Much lower process loading is required if wastewater contains suspended solids.<br>3. Special bio-reactor configuration is required which is based on experience.<br>4. Shorter bio-reactor HRTs mean less equalization and dilution of inhibitors. |

model was proposed to define the effect of a limiting substrate or nutrient.

### 2.7.1 Monod Equation

Mass balance equations for microorganisms and limiting substrate in a continuous flow system can be estimated by equating accumulation against the increases and decreases occurring in an infinitely short time interval as follows:

| Rate of accumulation of microorganism within the system boundary | = | Rate of flow of microorganism into the system boundary | - | Rate of flow of microorganism out of the system boundary | + | Net growth of microorganism within the system boundary |
|---|---|---|---|---|---|---|

This can be written as:

Accumulation = Inflow - Outflow + Net growth

The rate of change of biomass in the system as mentioned above can be expressed as:

$$\frac{dX}{dt} = \left(\frac{Q}{V}\right)X_0 - \left(\frac{Q}{V}\right)X + \mu X - K_d X \qquad (2\text{-}8)$$

Where

Q = the flow rate, L/day

V = volume of the reactor, L

$X_0$ = concentration of biomass in the feed, g VSS/L

X = concentration of biomass in the reactor, g VSS/L

M = specific growth rate, $day^{-1}$

$K_d$ = death rate constant, $day^{-1}$

The specific growth rate ($\mu$) is a measure of how quickly the cell population is growing. The higher the value of $\mu$, the greater the rate of the growth.

Monod equation states that:

$$\mu = \frac{\mu_m S}{(K_s + S)} \qquad (2\text{-}9)$$

Where

S = concentration of the limiting substrate (g/L)

$\mu_m$ = maximum specific growth rate ($d^{-1}$)

$K_s$ = the saturation constant (g/L)

The rate of change in substrate concentration in the reactor could be expressed as:

$$\frac{dS}{dt} = \left(\frac{Q}{V}\right)S_0 - \left(\frac{Q}{V}\right)S - \frac{\mu X}{Y} \qquad (2\text{-}10)$$

Where

$S_0$ = substrate concentration in feed, g COD/L

Y = yield coefficient, g VSS/g COD

### 2.7.2 Contois equation

Other kinetic model was also developed based on the Contois equation. The relationship between the specific growth rate and the rate limiting substrate concentration can be expressed as follows:

$$\mu = \frac{\mu_m S}{(B + S)} \qquad (2\text{-}11)$$

Where

B = the kinetic parameter (g COD/g Biomass)

### 2.7.3 Kinetic Coefficients of Anaerobic Process

The kinetic parameter estimation has been carried out by several researchers. Metcalf & Eddy (1991) summarized the rate constants values of anaerobic process for treating various types of wastewater as shown in Table 2.8. The maximum of Y value was observed in protein type wastewater with the average value of $0.075 \frac{mgVSS}{mgBOD_5}$. Jeyaseelan (1997) summarized the values of kinetic constants in anaerobic process for each substrate component and various temperatures as illustrated in Table 2.9. The obtained results indicated that the decreasing of

temperature caused the increasing of $K_S$ Nopharatana et al. (2003) also developed a dynamic mathematical model for sequential leach bed anaerobic digestion of organic fraction of municipal solid waste and used hydrolysis kinetics that have been reviewed by various investigators for each type of anaerobic bacteria as shown in Table 2.9. The minimum value of $K_s$ was reported for hydrogen utilizing methane bacteria.

In addition, Hu et al. (2002) studied the anaerobic digestion of ice-cream wastewater, and the kinetic parameters were estimated by using both Monod and Contois model, as illustrated in Table 2.10. The Contois-type model was found to be more suitable than the Monod type, particularly the microbial kinetics of anaerobic digestion treating fat-rich wastewater.

**Table 2.8** Typical kinetic coefficients for the anaerobic digestion of various substrates reported by Metcalf & Eddy (1991).

| Wastewater Type | Coefficient | Value | |
|---|---|---|---|
| | | Range | Typical |
| Domestic sludge | Y | 0.040 – 0.100 | 0.06 |
| | $k_d$ | 0.020 – 0.040 | 0.03 |
| Fatty acid | Y | 0.040 – 0.070 | 0.05 |
| | $k_d$ | 0.030 – 0.050 | 0.04 |
| Carbohydrate | Y | 0.020 – 0.040 | 0.024 |
| | $k_d$ | 0.025 – 0.035 | 0.03 |
| Protein | Y | 0.050 – 0.090 | 0.075 |
| | $k_d$ | 0.010 – 0.020 | 0.014 |

Remarks: The unit of Y value is mg $\frac{mgVSS}{mgBOD_5}$

The unit of $k_d$ is $d^{-1}$

**Table 2.9** The kinetic parameters of anaerobic wastewater treatment process reviewed by Jeyaseelan (1997) and Nopharatana et al. (2003).

| Component | Y | k ($d^{-1}$) | $K_s$ (mg/L) | $k_d$ ($d^{-1}$) | Reference |
|---|---|---|---|---|---|
| Acetic acid 35°C | 0.04 | 2.1 | 154 | 0.019 | Jeyaseelan (1997) |
| 30°C | 0.054 | 4.8 | 333 | 0.037 | |
| 25°C | 0.05 | 4.7 | 869 | 0.011 | |
| Propionic acid 35°C | 0.042 | 9.6 | 32 | 0.010 | |
| 25°C | 0.051 | 9.8 | 613 | 0.040 | |
| Butyric acid 35°C | 0.047 | 15.6 | 5 | 0.027 | |
| Fatty acid 35°C | 0.12 | 6.67 | 680 | 0.015 | |
| 30°C | 0.12 | 4.65 | 1270 | 0.015 | |
| 25°C | 0.12 | 3.85 | 1580 | 0.015 | |
| Glucose 37°C | 0.173 | 30 | 23 | 0.8 | |
| Acedogenic bacteria | 0.204 | 11.77 | 500 | 0.048 | Nopharatana et al. (2003) |
| Aceticlastic methane bacteria | 0.023 | 6 | 360 | 0.101 | |
| Hydrogen utilizing methane bacteria | 0.159 | 28.9 | 30 | 0.048 | |

**Table 2.10** Kinetic parameters investigated by Hu et al. (2002).

| $\mu_{max}$ ($day^{-1}$) | | $K_s\left(\frac{gCOD}{L}\right)$ | $B\left(\frac{gCOD}{gVSS}\right)$ | $Y\left(\frac{gVSS}{gCOD}\right)$ | $K_d$ ($day^{-1}$) |
|---|---|---|---|---|---|
| Monod | Contois | | | | |
| 0.7844 | 0.9297 | 0.4028 | 0.4818 | 0.2116 | 0.0131 |

## 2.8 Overview of Mathematical Models

The management of wastewater treatment involves complex processes due to many interacting parameters, some of which are difficult to present in a straightforward mathematical model, equation, or formula. Mathematical modeling techniques can be used to aid in predicting the quality and sequence of relationships to

solve the problem. A model can be regarded as an assembly of concepts in the form of one or more mathematical equations that approximate the behavior of a natural system or phenomena. Simulation models address the formulation of a mathematical model that simulates a specific situation, with the development of mathematical relationships and solution through a structured and valid process.

### 2.8.1 Technique Used for Developing Mathematical Model

One objective in this study was to construct a model to represent the process of dairy wastewater treatment that might be used as a tool for assessing the feasibility of waste water treatment by UASB process and predicting the methane formation for long-term energy management. Due to the complex nature of wastewater treatment itself, the proposed model should consist some specific characteristics: non-linear dynamics, extensive feedback mechanisms and it should be interactive and readily responding to the changes of wastewater characteristics and treatment conditions. For the reasons, the system dynamics simulation technique was employed.

### 2.8.2 Overview of System Dynamics Simulation Technique

System dynamics is a subset of the large field of simulation modeling and was developed at Massachusetts Institute of Technology (MIT) during the 1950s, by Jay W. Forrester who brought together the ideas from new-control engineering, cybernetics and organizational theory (Keerativiriyaporn, 1998). From these basic ideas, a set of representational techniques for simulating complex, non-linear and feedback-rich system was developed and has grown to provide a single framework for understanding the behavior of electronic, chemical, biological, and social systems whose elements interact through time to produce system changes.

### 2.8.3 Typical Characteristics of System Dynamics Models

System dynamics models are formulated based on the assumption that any change in a system is caused by a feedback structure and interactions between elements in the system. They usually have the following characteristics.

1) Most of variables in system dynamics models occur in feedback relationships and are mostly endogenous. Factors influencing the systems from outside without being influenced by other factors in the model are represented as exogenous variables.

2) Due to feedback emphasis, the models are made up of many loops linked together and basically close system representations.

### 2.8.4 Processing of System Dynamics Simulation

The proposed model was developed by the steps of the system dynamics simulation technique (Richardson and Pugh, 1981) outlined as follows:

1) Problem identification and definition: Problem identification is the initial step prior to do any other steps in system simulation process. In this stage, the characteristics of reactor digestion is dynamic or changes over time then, the problems of dairy wastewater treatment process will be identified and addressed by means of literature review.

2) System conceptualization: At this stage, it will include the process of defining problems dynamically and representing the feedback structure of a model with causal loops and flow diagrams. Dairy wastewater problem, cause and its relationship will be defined verbally and graphically. The relationships of various variables involving in dairy wastewater treating process and by-product of biogas will be bound together in the form of causal loops and flow diagrams.

3) Model representation: At this stage, it will include the translation of the flow diagrams into mathematical forms, the assignment of values and the specification of model's empirical functions.

4) Analysis of model behavior and model evaluation: This stage deals mainly with the simulation runs of developed mathematical model and calibrated with the results obtaining from experiment. This is done by carrying out a sensitivity analysis on developed model.

### 2.8.5 Computer Language Used in Mathematical Model

STELLA is a graphical programming language developed by High Performance Systems, Inc. (High Performance System, Inc., 2000) specifically for system dynamics study. As a graphical programming language, it allows a modeler using the program's graphical tools and functions to build dynamic models (High Performance System Inc, 2000). STELLA is a program using an interactive model procedure that can be presented either graphically or as a list of equations. Graphic output possibilities are excellent (Dunn et al., 1988).

Although the system dynamics model can be written either in general-purpose language such as PASCAL, BASIC or FORTRAN, due to the ability as mentioned above and the convenience of model building, STELLA was employed for this study.

### 2.8.6 Applications of STELLA in Various Fields

Duplisea (1998) used STELLA software to study the feedbacks between benthic carbon mineralisation and community structure by constructing an ecological simulation model. Model simulations generated reasonable results and compared with the empirical data from benthic systems. Martin and Reddy (1997)

studied the interaction and spatial distribution of wetland nitrogen processes by means of simulation analysis. Most processes were represented by first-order kinetics, except vegetative uptake, which was represented by Michaelis-Menten kinetics. A spatially explicit, two-dimensional model was developed to evaluate the processes which determine the fate and transport of nitrogen (N) in wetland system. STELLA inconographic software was used to simulate processes regulating N removal from wetlands. Krivtsov et al. (2000) employed system dynamics approach to study the indirect regulation rule for consecutive stages of ecosystems, management of natural resources, environmental assessment and auditing. Lindgren (1998) studied climate change, tick-borne encephalitis and vaccination needs in Sweden- a prediction model presented an example of a modeling tool for projections of possible changes in the incidence of tick-borne encephalitis (TBE), and the subsequent changes in vaccination needs, during the next half-century in Sweden. The model has been constructed into STELLA. According to the constructed model, the annual vaccination rate needs to increase by 3-4 fold during the next half century in order to prevent the projected increases in TBE incidence in the region from a climatic change. The obtained results would be beneficial in health system in Sweden. Woodwell (1998) used STELLA to study a simulation model to illustrate feedbacks among resource consumption, production, and factors of production in ecological-economic systems. He indicated that the greatest value of the model was not in prediction or forecasting, but in revealing and developing our basic understanding of the relationships between the economy and the environment. Dynamic models in chemical and biological engineering are usually non-linear, and the resulting differential equations require numerical solution. In order to simulate the results, the graphical capabilities are

limited. Therefore, they used STELLA as a tool for application in chemical and biochemical engineering education and found that this computer simulation modeling methods enhanced the learning process.

Mesple et al. (1996) studied the modeling of orthophosphate evolution in a high rate algae pond by using STELLA software, it was thought that deterministic modeling of the temporal evolution of $PO_4$ might provide a rational basis for pond management policies.

Jamu and Piedrahita (2002) constructed a dynamic and mechanistic mass balance model by using STELLA for prediction of nitrogen and organic matter outputs from aquaculture ponds and their subsequent recycling in conventional agriculture practices. Data from Thailand, Honduras and Malawi were used for calibration. The structure of the model allowed users to modify parameter values to suit different simulation scenarios via a user interface display that also included graphs and tables for model output.

# Chapter III

# Research Methodology

In order to achieve the objectives as mentioned in Chapter 1, the overall conceptual frameworks of the experimental research was developed into 2 phases as shown in Figure 3.1.

## 3.1 Experimental Design

The experimental approach for this study can be categorized into 4 steps as follows.

### 3.1.1 Bio-reactors Preparation

Two identical UASB reactors were constructed and installed at Building 2 located in Department of Environmental Health Science, Faculty of Public Health, Mahidol University. The physical properties of reactors and built reactor are illustrated in simplified schematic diagram and in Figure 3.1 and Table 3.1.

Each reactor was made of acrylic pipe, with the internal diameter of 15 cm and 1.2 m height. The total volume was 20 liters but the effective volume was 16.8 liters. Five sampling ports were incorporated at the interval of 20 cm, along the reactor column. These sample ports were used for pH measurement of liquid containing in the reactor and for taking the sludge for size classification. The reactors were equipped with a proper gas-liquid-solid separator (GLS) in the upper inside part. When the mixed liquor

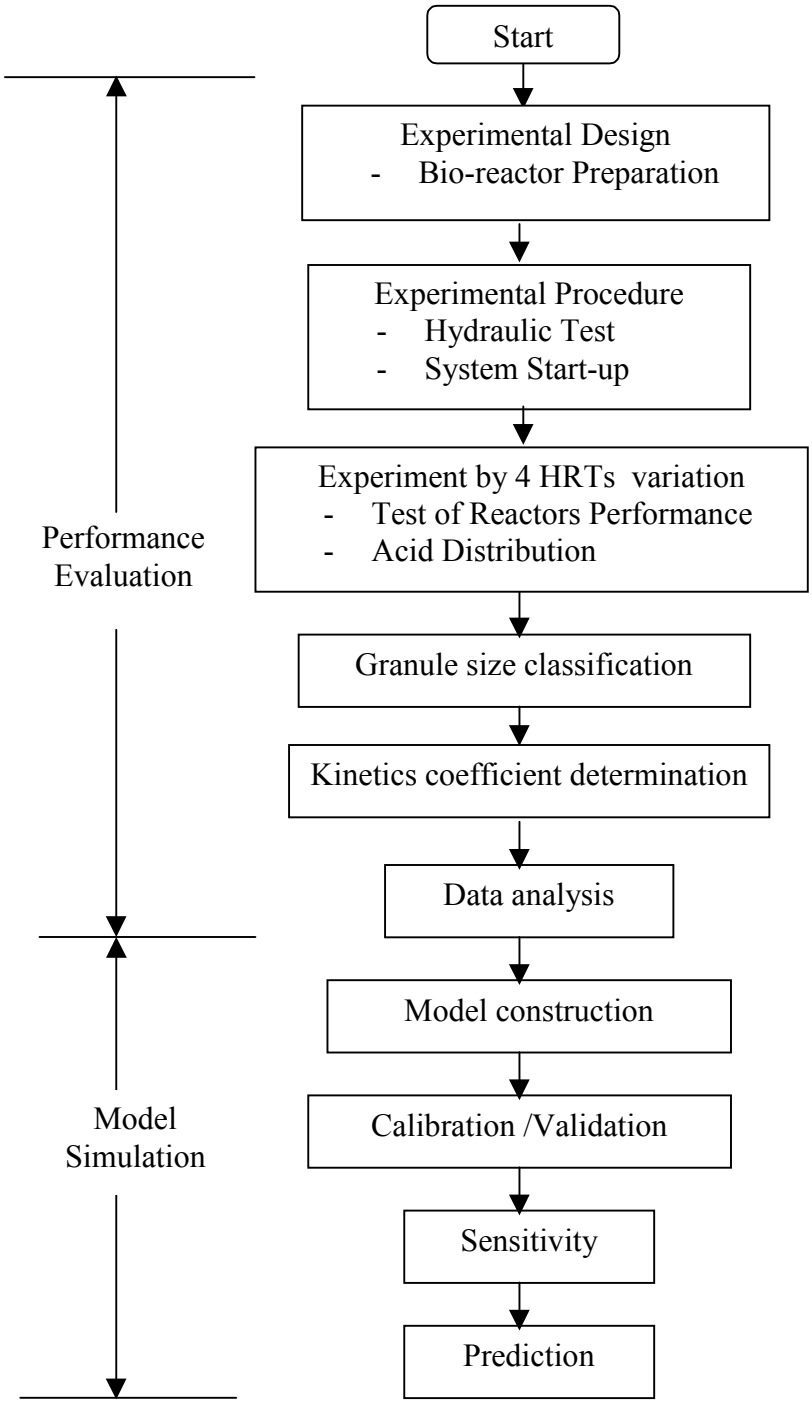



**Figure 3.1** The overall steps used in this study.

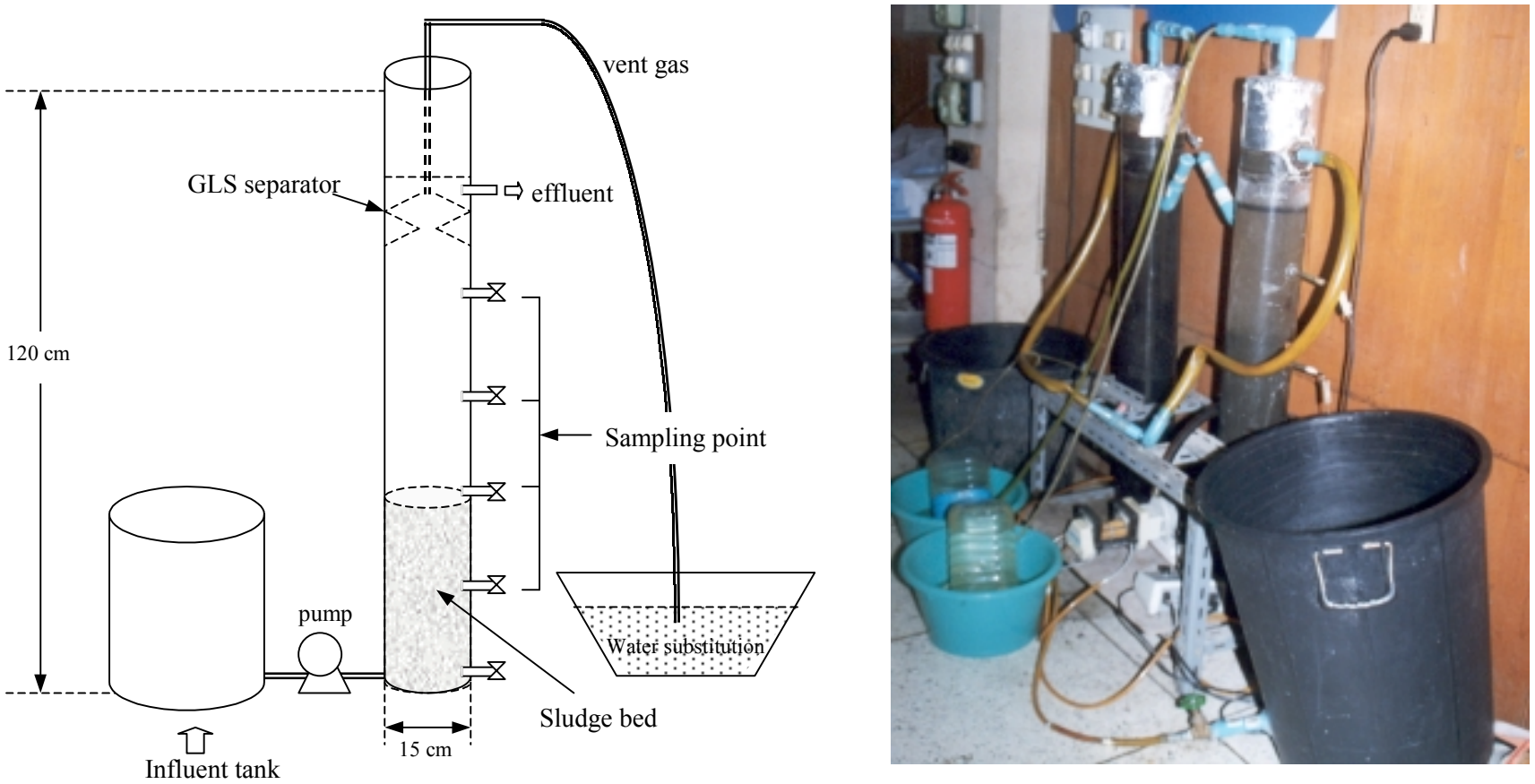



**Figure 3.2** Schematic diagram of UASB experimental set up versus built and used reactor for this study.

**Table 3.1** Characteristics of UASB reactors used for this study.

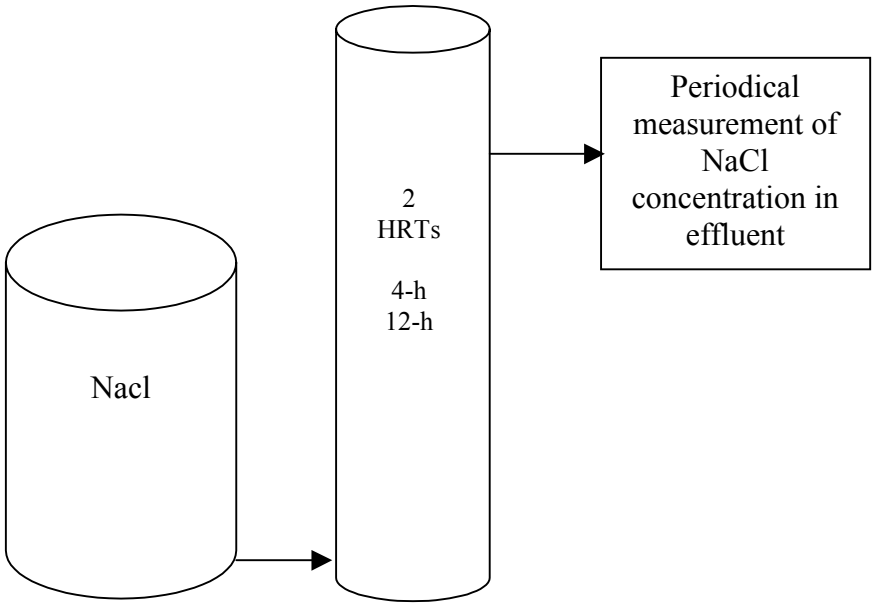
| Item | Size |
|---|---|
| 1. Material: Acrylic pipe | Inner diameter 15 cm.<br>Height 120 cm. |
| 2. Sampling outlets | 5 outlets with 20-cm interval |
| 3. Sludge: Pepsi wastewater treatment plant, Nonthaburi province | 9 Liter per reactor with MLVSS of 14,000 mg/L<br>Sludge height = 30 cm. |
| 4. Biogas volume measurement | Gas substitution in water |
| 5. Total effective volume | 16.8 liters |

arrived at this settler, the sludge particles were settled out and retained in the bottom part, the so-called digestion zone. The GLS separator was acrylic material with a height of 20 cm and inclined walls of approximately 45 degree slope.

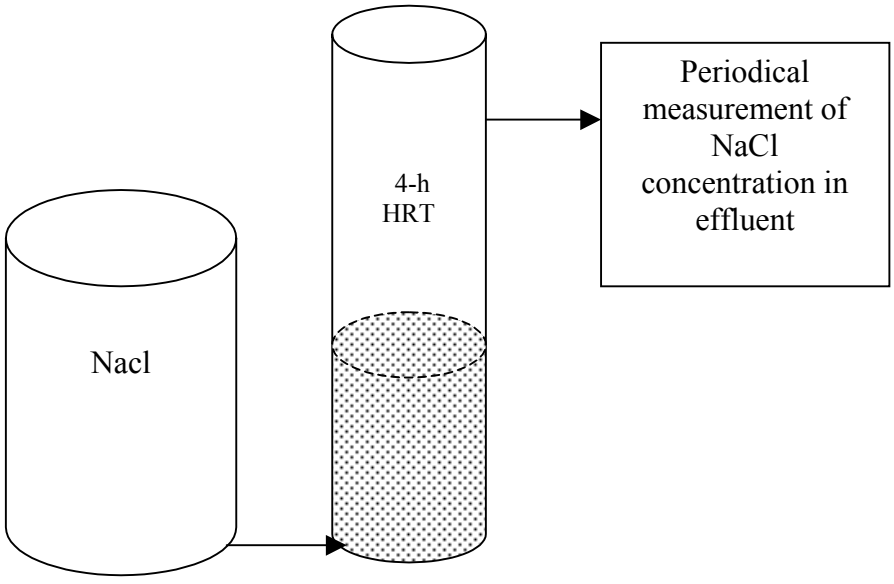
### 3.1.2 Experimental Procedures

The experimental steps can be divided into 3 parts as follows.

1) Hydraulic Test: Hydraulic test was performed by means of tracer study using sodium chloride of 50 g NaCl/L concentration, 20 liters of which was fed into the reactor with 70 mL/min or 4-h HRT and 33.6 mL/min or 12-h HRT for empty-bed reactor and 4-h HRT for sludge-containing reactor. The effluent concentrations, in terms of NaCl, were monitored every 10 minutes at the outlet. The procedure for hydraulic test is illustrated in schematic diagram in Figure 3.3. The dispersion number was determined by using Levenspiel method (Levenspiel, 1972) as the following equations.




(a) Empty-bed reactor




(b) Sludge-containing reactor

**Figure 3.3** The schematic diagram for hydraulic test of UASB reactor.

$$T_{mean} = \frac{\sum_{i=0}^{t} t_i c_i \Delta t}{\sum_{i=0}^{t} c_i \Delta t} \quad (3\text{-}1)$$

$$\sigma^2 = \frac{\sum_{i=0}^{t} t_i c_i \Delta t - T_{mean}^2}{\sum_{i=0}^{t} c_i \Delta t} \quad (3\text{-}2)$$

$$2d + 8d^2 = \frac{\sigma^2}{T_{mean}^2} \quad (3\text{-}3)$$

Where

$T_{mean}$ = average retention time, minutes

$\sigma^2$ = coefficient of variation

d = dispersion coefficient = $\frac{D}{\mu L}$ or dispersion number

2) System start-up: Firstly, anaerobic bacteria (seeding material) was fed into reactors. The seed was obtained from Pepsi wastewater treatment plant in Nonthaburi province. The synthetic dairy wastewater was prepared by using UHT milk diluted in trap water with the similar characteristics of dairy wastewater generated in Thailand and was fed with a peristatic pump continuously.

3) Test for reactors performance: According to the literature review as mentioned in Chapter II, in using UASB process to normally treat the wastewater, the designed HRTs ranged from 4-h to 48-h. Therefore, this experiments were conducted with 4 HRT variations of 12, 16, 20 and 24 hrs, which corresponded to the influent flow rates of 33.6, 25.2, 20.16 and 16.8 L/d, respectively. The synthetic dairy wastewater was daily prepared with COD concentration of 700 - 1,200 mg/L and

continuously fed into the bottom of reactors. The reactors performance was assessed everyday to determine the steady state in laboratory and was daily assessed during the steady state for 5 days for each HRT. The steady state condition was determined when the standard deviation values of removal efficiency were less than 5%. After the steady-state condition was reached, influent and effluent samples were taken for daily laboratory analysis with 3 samples per day totally 5 days to determine the parameters as presented in Table 3.2, following the Standard Methods (APHA et al., 1998). Then, there were totally 15 samples in each HRT. Figure 3.4 shows the schematic diagram for overall experiment and analysis used in this study.

4) Acid distribution: As mentioned before, there were 3 phases of anaerobic process namely; (I) hydrolysis, (ii) acidogenesis and (iii) methanogenesis. If the reaction in methonogenesis was not long enough, there would be some organic acids remained in the effluent. If the acetic acid was also monitored in the effluent, it indiacted that the period of methanogenesis was not sufficient to convert methane cursor or acetic acid to methane gas. Therefore, organic acid concentration in effluent were determined by using GC. In addition, the reaction rate for each step of anaerobic process were also determined.

5) Kinetics coefficient determination: The kinetic coefficients were calculated by using Monod equation.

The effect of upflow velocity on granule size: After the end of HRT the sludge granules were taken from reactor for size classification and finally the mean size of granule were statistically compared for all 4 HRTs. The hypothesis for

6) this study was follow, Ho: The averages of granule size were not different for all 4 upflow velocities and H1: The averages of granule size were

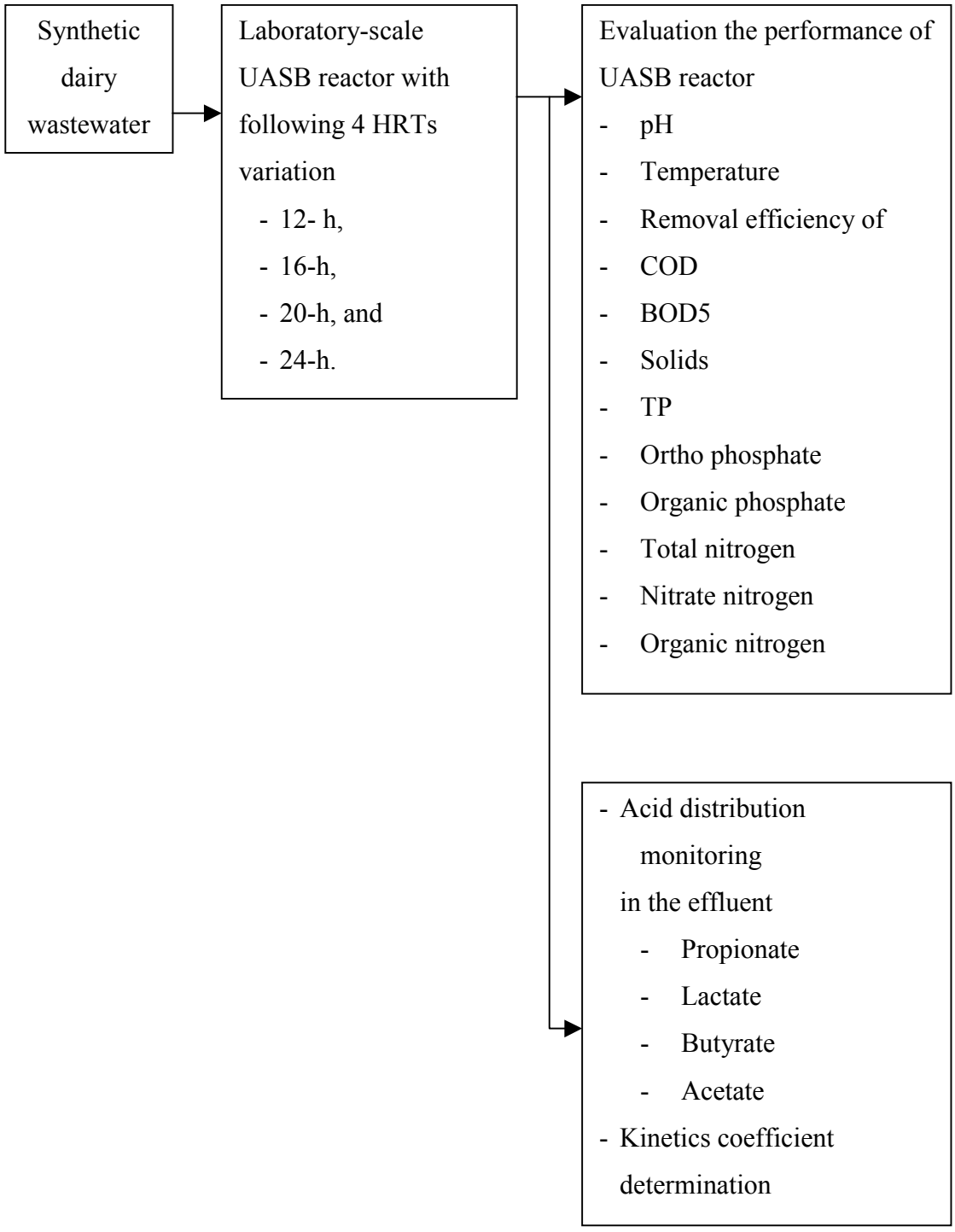



**Figure 3.4** The schematic diagram for performance evaluation of reactor.

**Table 3.2** The parameters and analytical methods for influent and effluent.

| Influent | Analytical methods |
|---|---|
| pH | pH meter |
| Temperature | Thermometers |
| COD | Closed Reflux, Titrimetric method |
| $BOD_5$ | Azide Modification |
| SS | Suspended Solids Dried at 103-105$^oC$ |
| VSS | Suspended Solids Dried at 550 $^oC$ |
| TP | Persulfate Digest/Ascorbic Acid Method |
| Ortho phosphate | Ascorbic Method |
| TKN | Kjeldahl Digestion |
| $NO_3^-$ | Brucine Method |
| $NH_3$-N | Titrimetric Method |

different for all 4 upflow velocities.

## 3.2 Mathematical Model Development

The methodological approach for mathematical model development was involved the following steps.

### 3.2.1 Causal Loop Diagram

Figure 3.5 shows the initial causal loop diagram of methane gas formation from anaerobic process that was used for simulation. The volume of

methane gas depends upon the various factors such as the content of nutrients in dairy wastewater in terms of COD or BOD, and the number of microorganism surviving in reactor. For example, the more nutrients in wastewater, the more acid distribution may occur and the more methane volume would formed.

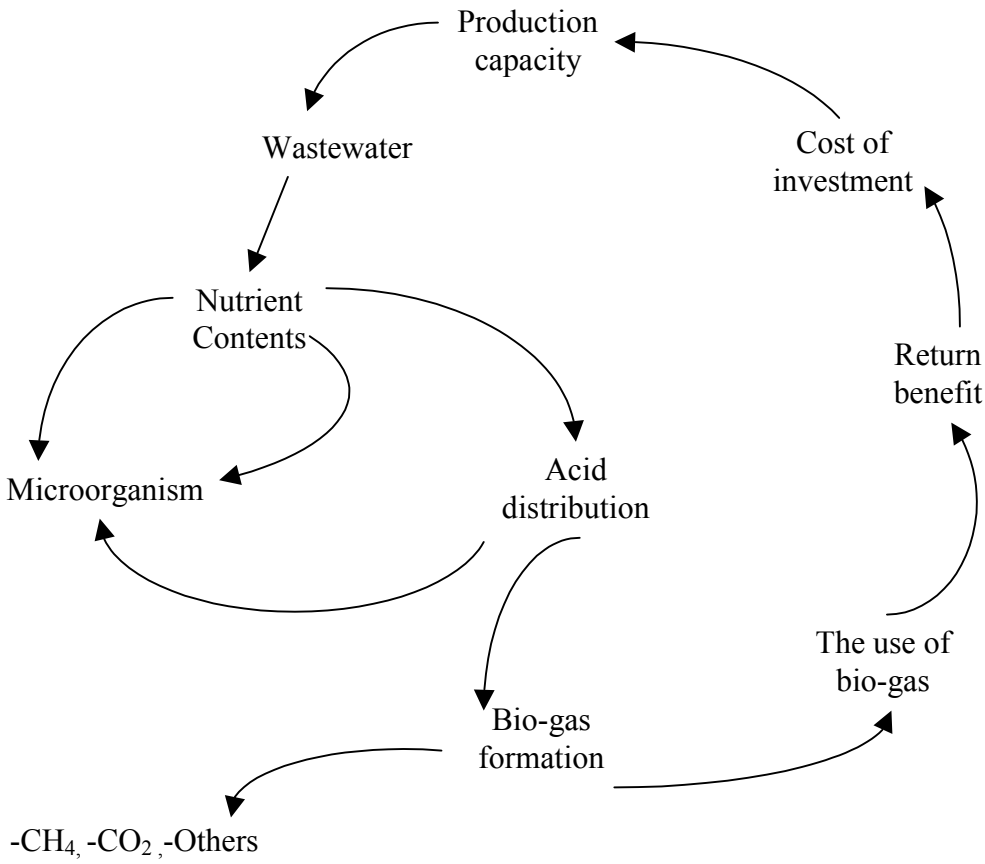



**Figure 3.5** The initial concept of causal loop for mathematical model development.

### 3.2.2 Equation Translation

The causal loop diagram shown in Figure 3.5, it was converted to flow diagram, which shows the interactions between the principal elements in form of

system dynamics flow diagram. The flow diagram format was used to represent the interactions in the proposed model. Four types of structure variables are used in flow diagram to present: level, rates, auxiliaries and clouds as shown in Figure 3.6.

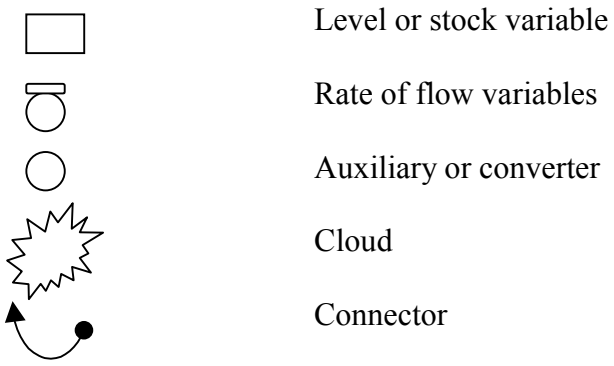



**Figure 3.6** The symbols used in flow diagram.

Level is the variable that accumulates its quantity over time. It means the quanity of level variable can be increased or decreased through time. Therefore, the unit of level variable generally is unit of amount or number e.g. number of microorganisms.

Rates or flow variable is depicted by valve form, as the model is running, rate will change the condition of levels. Rate is the activity, movement or flow of material per unit of time into or out of the level variable. Hence, unit of rate variable is the amount per unit of time e.g. volume of influent per second. The direction of flow is indicated by the arrowhead. For example the increasing of influent flow will cause the increasing of wastewater volume in reactor.

Auxiliaries or converters are represented by circles; they will convert

input to output.

Clouds are the level variables, which are dynamically unimportant and therefore placed outside the boundary of the model. They represent things that need not to be known exactly from where they come or will be terminated. The cloud is depicted by a cloud symbol.

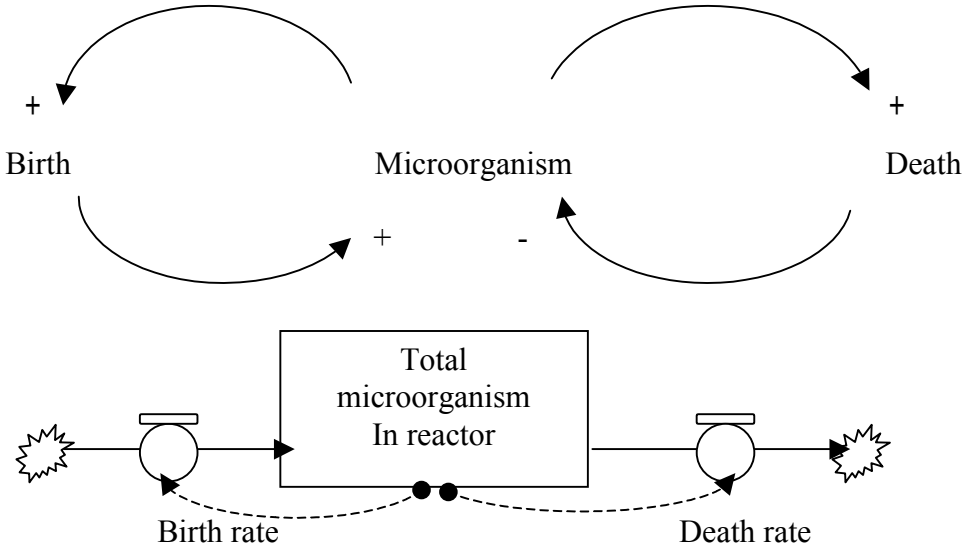
Connectors are illustrated in Figure 3.6. The system dynamic flow diagram symbols are connected by connectors, arrows with a small circle at the beginning of the arrows, reflecting the assumptions about 'what depends on what"

### 3.2.3 Model Simulation

Model simulation is the process of converting conceptual model into quantitative representation. It involves the translation of flow diagram into model structure, which consists of series of equation. The formulation of model in this study used STELLA software. Figure 3.7 is the example of converting causal loop into flow diagram and later to equation. The model consists of one level variable, which is total microorganism, and two rate variables i.e. birth rate and death rate. According to STELLA format, level equation which represents the calculation of total microorganism can be written as illustrated in Figure 3.7.

### 3.2.4 Validation and Sensitivity Analysis

When a model is formulated, normally there are questions concerned with the model such as "Is the model suitable for its purposes and consistent with the reality or real word it tries, to capture?" Therefore, in order to clarify the above questions, the evaluation should be performed with the following two processes namely validation and sensitivity analysis.




Total_microoganism(t) = Total_microoganism(t-DT) + (birth_rate – death_rate)*DT

**Figure 3.7** An example of causal loop, flow diagram and equation translation.

1) Model validation and calibration: Model validation was defined by Lemon (Jongkaewwattana, 1995) as "the comparison of a verified model with the real world and determination if it is suitable for its intended purpose". Forrester and Seng (1980) reported that validation is "comparison of the predictions of a verified model with experimental observations other than those used to build and calibrate the model, and identification and correction of errors in the model until it is suitable for its intended purpose".

In this study, the model validation and calibration procedure involved comparing the performance or the outputs of the model against recorded data or against a subjective judgement of the expected outputs, given a broad understanding

of the system being model. The data obtained from the first run (24-h HRT) was used for calibration and then the gained data from the second and third run (20-h and 16-h of HRT) were used for validation.

The output parameters (methane formation, removal efficiency, etc) from the model were then compared with the measured output from laboratory. The type of validation may be numerical validation and/or behavioral validation. Statistical test was employed for numerical validation by means of goodness of fit. In case of behavioral validation, a visual comparison of model prediction was compared with the laboratory results.

2) Sensitivity analysis: Sensitivity analysis is a procedure, which is normally performed on the completed and, at least partly validated model. It involves the exploration of the operation and performance of the model. That is, in the successive runs of the model under identical conditions, the value of a parameter is changed. Consequently, the outputs from the runs are then analyzed in order to determine whether the changed parameter values are of material consequence. The model, which would be a good representation of the real system, would produce a reasonable change of the outputs.

# Chapter IV

# Results and Disccusion

## 4.1 Hydraulic Test Study (Tracing Study)

Hydraulic performance of the reactor was tested for 2 cases according to the condition of reactor as empty-bed reactor and granular-sludge containing reactor.

### 4.1.1 Empty-bed Reactor

1) 4-h HRT: Calculation of concentration and time in the tracer study is tabulated in Appendix A. The average retention time ( $T_{mean}$ ) of empty-bed reactor for 4-h HRT was 282 minutes with dispersion number (d) of 0.056 whereas the theoretical retention time was 240 minutes, the concentration of sodium chloride versus time is shown in Figure 4.1. The value of this dispersion number was determined as intermediate amount of dispersion according to the Levenspiel's classification as indicated in Figure 4.2.

2) 12-h HRT: The average retention time ( $T_{mean}$ ) of this case was 1,022 minutes whereas the theoretical retention time was 720 minutes that indicated there was some minxing of sodium chloride and liquid contained in the reactor. Concerning the dispersion number, it was 0.0244 that can be determined as moderate dispersion number as shown in Figure 4.3.

### 4.1.2 Granular-sludge Containing Reactor

The average retention time ( $T_{mean}$ ) was 131 minutes as shown in

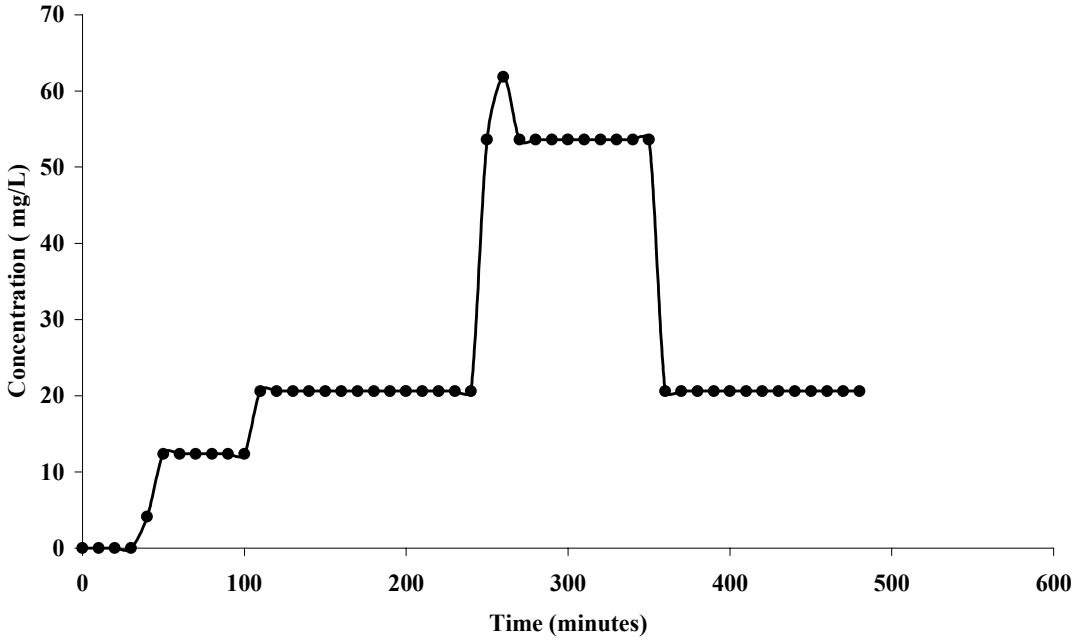



**Figure 4.1** The concentration of Sodium chloride ion versus time for tracer study of empty-bed reactor for 4-h HRT.

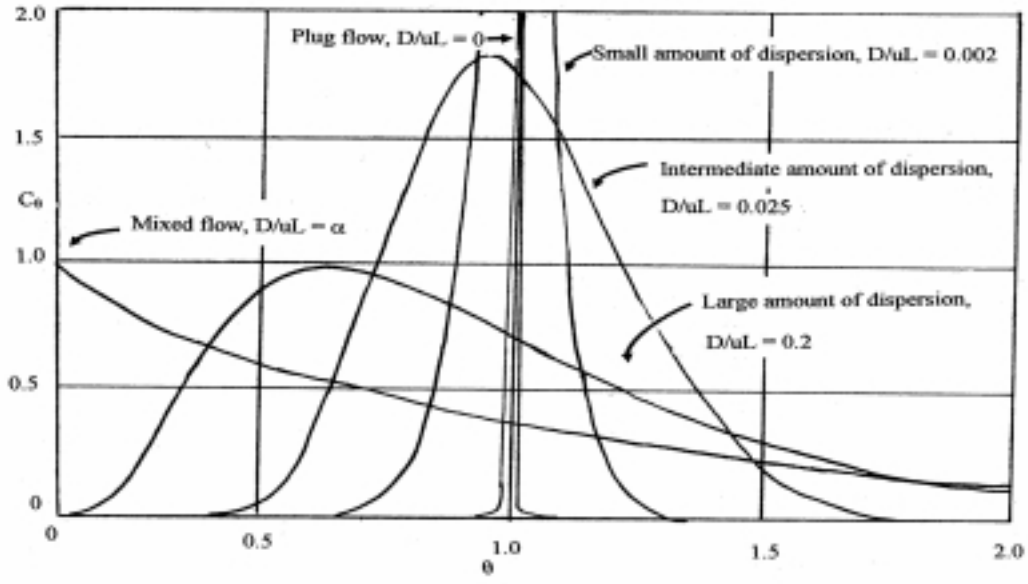



**Figure 4.2** Curves in closed vessels for various extents of back-mixing as predicted by the dispersion model.

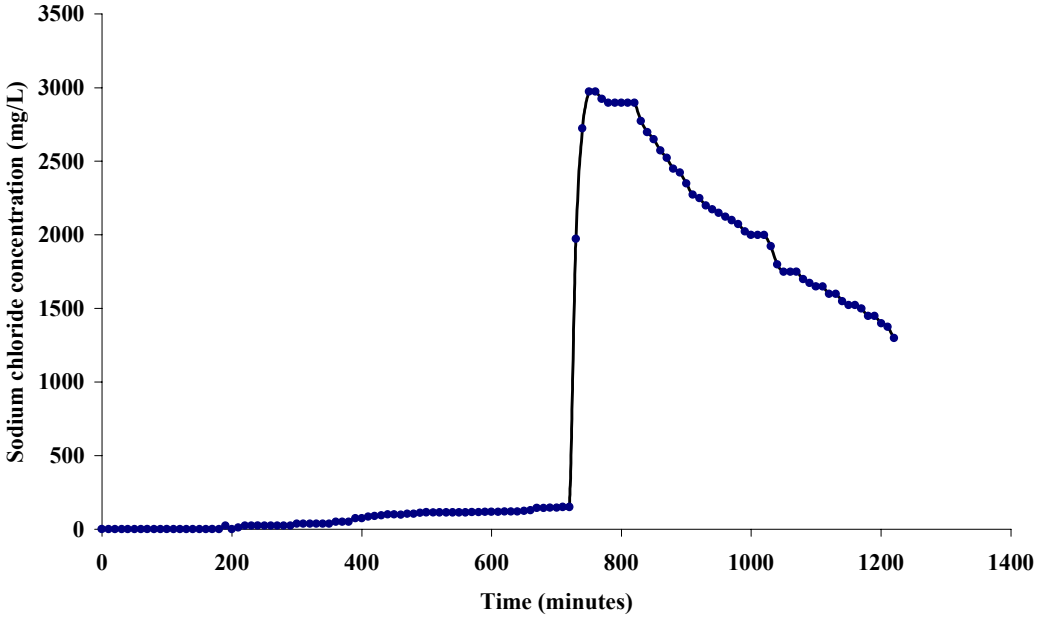



**Figure 4.3** The concentration of Sodium chloride ion versus time for tracer study of empty-bed reactor for 12-h HRT.

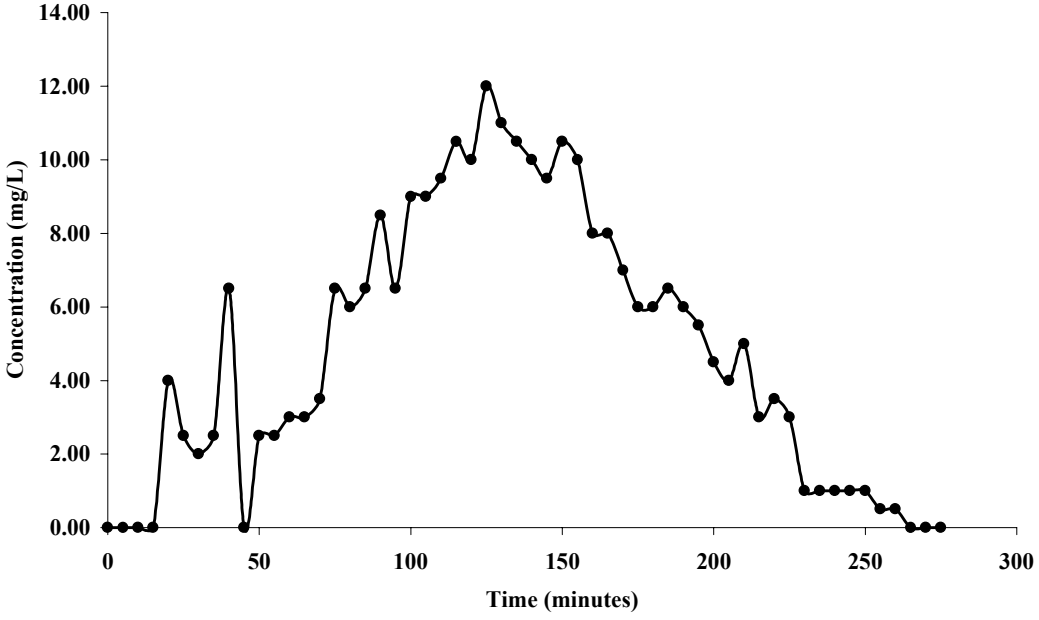



**Figure 4.4**. The concentration of Sodium chloride ion versus time for tracer study of granular-sludge containing reactor.

Figure 4.4, which is shorter than the theoretical retention time of 240 minutes. This was caused by the granule sludge contained in reactor, which obstruct the flow path of Influent. However, the estimated dispersion number of this case was slightly higher than the first case with the value of 0.059. However, it was also determined as intermediate level similar to the first case as illustrated in Table 4.1. If an estimated dispersion number exceed 0.2, a reactor contains a large dispersion number that means a reactor approximates to a single continuous tank reactor (CSTR) indicating a high degree of longitudinal mixing (Burrows et al., 1999). This result indicated that hydraulic test for both types of reactor were determined as intermediate dispersion.

**Table 4.1** The estimated dispersion number of empty-bed reactor and granular-sludge containing reactor compared with Levenspiel's classification.

| Item | Value of dispersion number, D/μL |
|---|---|
| Type of reactor used in this study | |
| Empty-bed reactor for 4-h HRT | 0.056 |
| Empty-bed reactor for 12-h HRT | 0.024 |
| Granular-sludge containing reactor | 0.059 |
| Levenspiel's classification | |
| Plug flow | 0 |
| Small amount dispersion | 0.002 |
| Intermediate amount of dispersion | 0.025 |
| Large amount of dispersion | 0.2 |
| Mixed flow | α |

Source: Levenspiel, 1972

## 4.2 Start-up and Initial Stage of Experiments

The UASB reactor was initially fed with anaerobic sludge with an amount of 4,000 mg VSS/L. The initial hydraulic loading applied was 0.0168 $m^3$/d and organic loading (OLR) were 1.01 - 2.07 kg COD/$m^3$-d. The synthetic dairy wastewater was fed continuously to reactor by a peristatic pump. The temperature of influent was maintained at ambient temperature, whereas the initial pH was approximately 6.7-7.5 and then it dropped to minimum value of 5.3. At the initial stage (during the first and second day of experiment) some sludge was washed out from reactor, but most of it was retained in the reactor due to the obstruction by the Gas-Liquid-Solid (GLS) separator and the gravitational settling. The bio-gas generated from the reactor was stored in a gas collection vessel that worked on the water displacement principle and it was observed to be in small quantity during the initial stage.

## 4.3 Influent Characteristics

The characteristics of synthetic dairy wastewater used in this study are illustrated in Table 4.2. It can be said that this wastewater is a low-strength type that has sufficient nutrients for anaerobic bacterial growth. The volatile suspended solids were approximately 20% of total solids which indicated a low fraction of organic matter in the form of suspended solids. Consequently, soluble organic matter in terms of total dissolved solids (TDS) existing in the wastewater was amendable to anaerobic process with the proportion of approximately 80% of total solids. The ratio of COD:N:P of 100:2.3:0.11 in the wastewater indicated that there were adequate nutrients for cell growth requirements. Comparing with characteristics of dairy wastewater studied by Gavala et al. (1999), the ratio of COD:N:P was 100:1.38:0.47.

**Table 4.2** Characteristics of synthetic dairy wastewater for this study taken from 15 samples.

| Parameters* | Range | Mean± Standard Deviation |
|---|---|---|
| BOD | 585 – 851 | 649±134.81 |
| COD | 853 – 1007 | 937±97.1 |
| pH | 5.3 – 7.5 | 7.0±1.1 |
| Temperature | 28 – 32 | 29.5±2.0 |
| SS | 127 – 290 | 194±80.6 |
| TDS | 525 - 948 | 794±201.0 |
| TS | 755-1075 | 988±155.8 |
| VSS | 56 – 260 | 131.1±88.9 |
| TP | 0.46 -1.84 | 1.04±0.54 |
| Ortho Phosphate | 0.34 – 1.20 | 0.7±0.36 |
| TKN | 18.37 – 27.23 | 21.57±4.23 |
| Nitrate Nitrogen ($NO_3^-$-N) | 0.11 – 0.88 | 0.645±0.37 |
| Ammonia Nitrogen ($NH_3^-$-N) | ND | 0 |
| Organic Nitrogen | 18.37 – 27.23 | 21.57±4.23 |

Remarks: ND = Not detecable

* unit in mg/L; except that of temperature, in $^oC$ and pH, dimensionless.

The proportion of nitrogen for this synthetic dairy wastewater was slightly higher than the Gavala's study. The value of BOD:COD of this synthetic dairy wastewater was 69:100, comparing to the characteristics of dairy wastewater in USA, BOD:COD was 51:100. The proportion of BOD to COD of this study was slightly higher than the study reported by Panesar et al. (1999). Regarding to the characteristics of actual

dairy wastewater generated from other plants in Thailand as illustrated in Table 4.3. It indicated that the BOD:COD ratio of this synthetic dairy wastewater closed to the wastewater of Suranaree University of Technology (SUT). Whereas, the average COD concentration was slightly lower than SUT dairy wastewater, the average concentrations of nitrogen and phosphorus of this synthetic dairy wastewater were higher than SUT dairy wastewater. Therefore, the ratio of COD:N:P of this synthetic dairy wastewater was higher with the value of 100:2.3:0.11, while the COD:N:P of SUT dairy wastewater was 100:1.62:0.02.

## 4.4 Reactor Performance

### 4.4.1 pH and Temperatures

The pH values of influent were in the range of 5.5 – 7.5 and temperature 28 – 33$^{o}$C. The pH values of effluent were in the range of 6.8 – 7.2 with the temperature ranging from 28 - 31$^{o}$C. The temperature of effluent in this study could be considered to be in mesophilic range (20-40$^{o}$C). The variation in temperature from upper to lower limit in this study (3$^{o}$C) did not have any influence on the performance of anaerobic bacteria. The lower limit temperature was 28$^{o}$C which is still within the suitable temperature condition for anaerobic bacteria since the decay rate of anaerobic bacteria is very low at temperature below 15 $^{o}$C (Rajeshwari et al., 2000). Regarding the influent pH of this study, the range of 6.8-7.2 are optimal for methane producing bacteria. Furthermore, effect of temperature and pH control on COD reduction was reported by the same researcher that if the pH and temperature of reactor were controlled, the more COD reduction was achieved, compared with the uncontrolled system.

**Table 4.3** Characteristics of this synthetic dairy wastewater used in this study comparing with dairy wastewater generated from Suranaree University of Technology and Kasetsart University dairy plants.

| Parameter | This study | | Suranaree* University of Technology | | Kasetsart University** | |
|---|---|---|---|---|---|---|
| | Range | Typical | Range | Typical | Range | Typical |
| COD, mg/L | 853 – 1,007 | 937 | 854 – 1,466 | 1,203 | 1,000 - 15,000 | - |
| BOD, mg/L | 585 - 851 | 649 | 600 - 960 | 791 | 500 – 1,000 | - |
| TKN, mg/L | 18.37 - 27.23 | 21.57 | 12 - 25 | 19.5 | 16 - 43 | - |
| TP, mg/L | 0.46 - 1.84 | 1.04 | 0.2 - 0.26 | 0.23 | 15 - 23 | - |
| BOD/COD, dimensionless | 0.69 | | 0.66 | | 0.53 | - |
| COD:N:P, dimensionless | 100:2.3:0.11 | | 100:1.62:0.02 | | - | - |

Note : - = not available

Source: * Lawanwattanakul (2002)

** Usakorn (1992)

### 4.4.2 Removal Efficiency

**1) COD Removal:** In this study, the soluble COD in the wastewater was approximately 72% of total COD, whereas its proportion in effluent was approximately 48%. The followings are the results obtained from laboratory analysis.

**a) COD removal with hydraulic retention time (HRT):** The total COD removals for 12-h to 24-h HRT's were in the range of 66.0 to 92.3%. The COD removal efficiency of 20-h HRT was slightly higher than 24-h HRT and slightly decreased at 16-h HRT. It can be observed that total COD removal clearly dropped at 12-h HRT as illustrated in Figure 4.5. Comparing with the study reported by Rajeshwari et al. (2000), a treatment efficiency of 90% was achieved with maximum organic loading rate (OLR) of 6.5 kg COD/$m^3$-d for treatment of dairy wastewater with COD of 2.05 g/L by using 10.7 $m^3$ UASB reactor. The removal efficiency of COD as mentioned above is not different from this study. In addition, results of this study showed that the variation of HRT (16-h to 24-h) had little effect on the COD removal efficiency. This was consistent with previous results recorded by Fang et al. (2000) on the effect of HRT on Mesophilic acidogenesis of dairy wastewater.

**b) COD removal with organic loading rate (OLR):** The total COD removal efficiency decreased slightly with an increase in organic loading rate (OLR) from 1.01 to 1.28 kg COD/$m^3$-d with the percentage of 92.3 to 90.1. Whereas the removal efficiency of COD at the OLR of 2.07 kg COD/$m^3$-d sharply decreased to 66%, as shown in Figure 4.6. In a similar study reported by Rajeshwari et al. (2000), the COD reduction of 90% dropped to 70-80% with the increase in organic loading rate from 6.5 kg COD/$m^3$-d to 45 kg COD/$m^3$-d for treatment of dairy wastewater with COD of 2.05 g/L by using 10.7 $m^3$ UASB reactor.

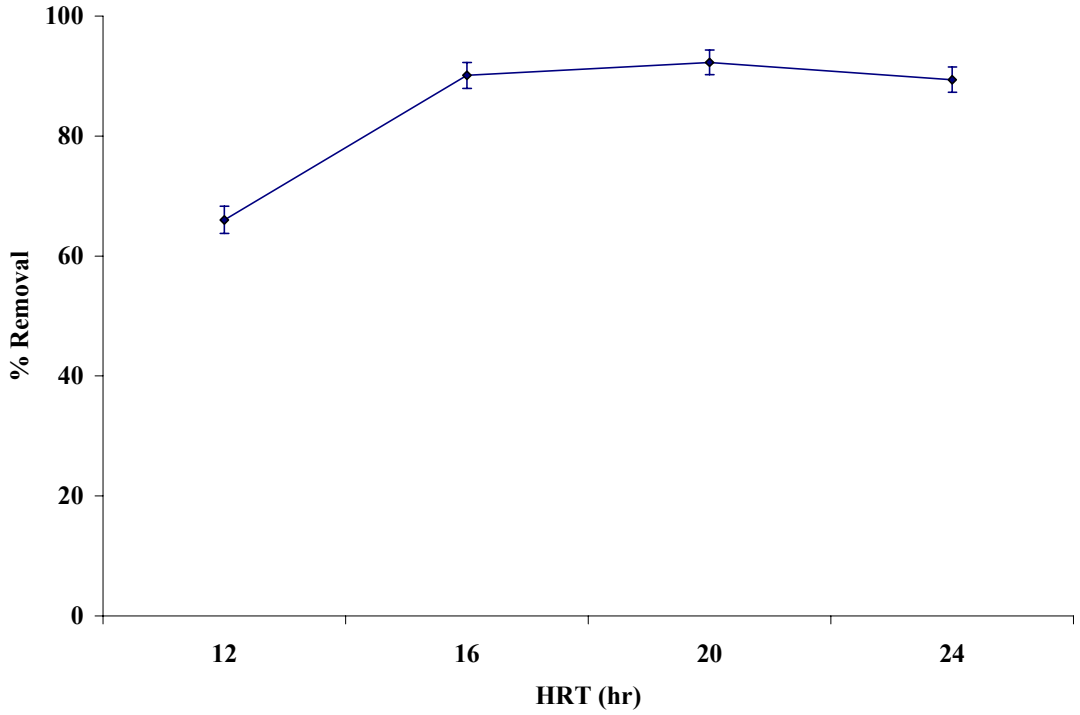



**Figure 4.5** The removal efficiency of total COD at various hydraulic retention times.

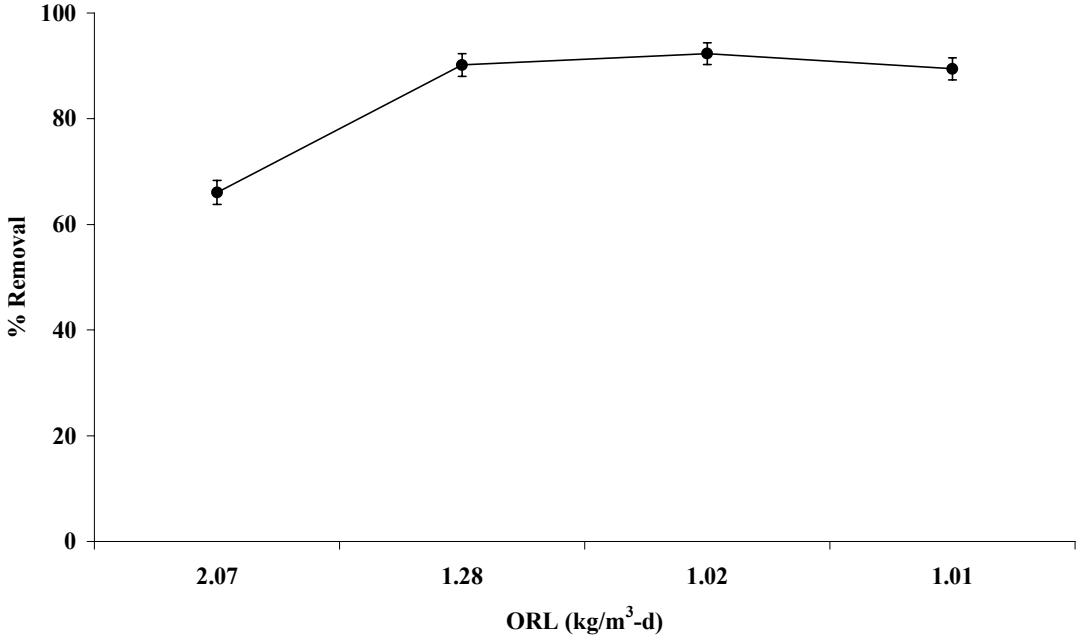



**Figure 4.6** Total COD removal for each organic loading rate (OLR).

Fang et al. (1995b) also indicated that COD removal efficiency of a UASB reactor is dependent on the COD loading rate. Panesar et al. (1999) reported that at one point of increased OLR for a UASB reactor treating dairy wastewater, lower performance was observed. Gavala et al. (2000) used UASB reactor treating dairy wastewater and also had the similar results. When increasing the influent COD concentration from 37 g/L or equaivalent to OLR of 6.2 g COD/L.d to 42 g/L or 7.5 g COD/L.d of OLR, the COD removal efficiency was reduced from 90 to 85%. After this point, the increase of OLR resulted in even lower efficiencies of COD removal. Thus, the removal efficiencies of COD observed by various researchers as mentioned above were not different from this study. This is a common problem encountered with cheese, whey or dairy wastewater, that when the substrate loading is increased, the acidogenic region extends into the methanogenic. The result of this is the poor efficiency in methanogenic phase of acidified wastewater and then the failure of the reactor would be observed. This indicates that the COD removal is related to OLR.

**c) Effect of Food to Microorganisms (F/M) Ratio on COD removal:** The influence of Food to Microorganisms (F/M) ratio on COD removal is illustrated in Figure 4.7. The F/M ratio was estimated on the basis of average COD loading fed at each hydraulic retention time and the amount of sludge measured at the end of steady state of each hydraulic retention time. Total COD removal efficiency varied from 66 to 92% with in the range of 0.37 to 0.73 g COD/g VSS-d of F/M ratio. As the F/M ratio was increased, the less removal efficiency of COD was observed. A similar result was reported by Wajanawijai (1991) who conducted the treatment brewery wastewater using UASB process and found that COD removal efficiency decreased from 94 to 90% while the F/M value varied from 0.35 to 0.95 g COD/g

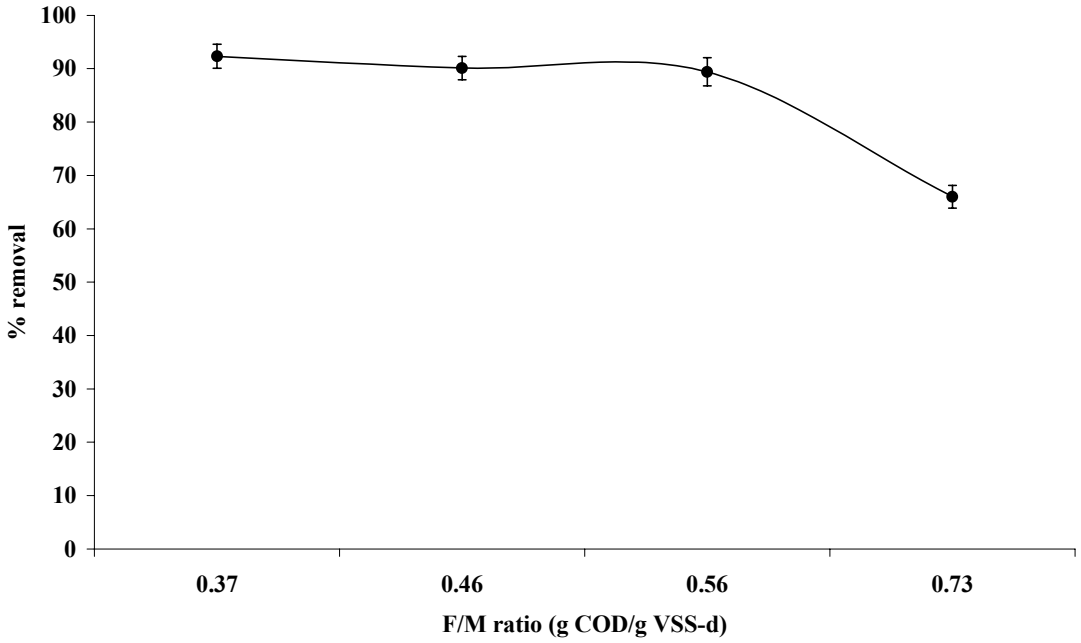



**Figure 4.7** The relationship of % removed COD and F/M ratio (g COD/g VSS-d).

VSS-d. Thus, the F/M ratio can be used as a parameter for testing the performance of UASB, high COD reduction (greater than 85%) can be achieved at proper F/M ratios, which could be in the range of 0.3 to 0.6 g COD/g VSS-d. Similar results were reported by Perez et al. (2001). A good COD reduction (96.6%) was achievable upto the F/M ratio of 0.55 kg COD/kg VSS-d. The COD removal decreased from 96.6 to 8.15% with the increasing F/M ratio from 0.04 to 0.55 g COD/g VSS-d, respectively.

**2) Solids Loss From the System**

The removal efficiencies of total solids (TS) are illustrated in Figure 4.8. The maximum removal efficiency was achieved at 24-h hydraulic retention time (HRT). This result indicated that the increased upflow velocity or shorter HRT caused the solids loss from the reactor or decreased the removal efficiency. Regarding to suspended solids (SS), the proportions of SS to TS were 12-

30% and 7-15% for times, influent and effluent, respectively. The proportion of VSS to TS were in the ranges of 7.4-24.5% and 5.7-23% for influent and effuent, respectively, which were the small fraction of total solids. It could be observed that this dairy wastewater mainly composted of dissolved organic matter since the proportions of TDS to TS were high level with the ranges of 69-88 and 85-92% for influent and effluent, respectively.

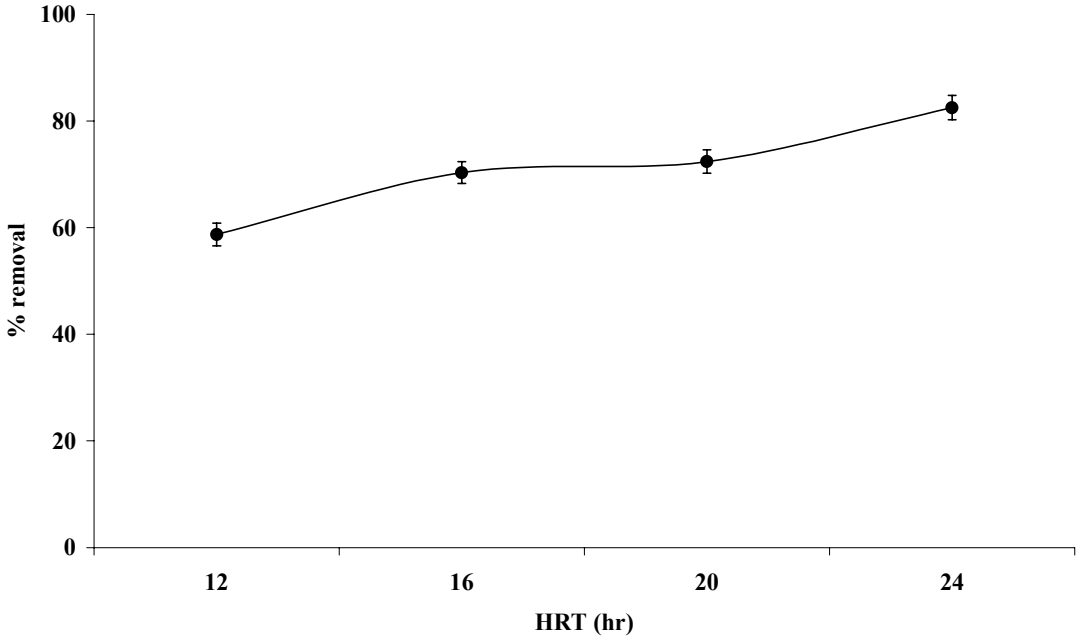



**Figure 4.8** The removal efficiencies of total solids at various hydraulic retention.

The volatile dissolved solids (VDS) for this dairy wastewater were slightly high with the proportions of 54-73% to total solids with the mean value of 67%. The results obtained from this study indicated that the removal efficiencies of solids for 16-h, 20-h and 24-h HRT were not significantly different and they clearly dropped at the 12-h HRT. Solids discharged in terms of total solids, volatile

suspended solids and total dissolved solids were effected by organic loading rate, and hydraulic retention times applied. Initially, the washout of sludge was higher due to the poor settleability of seed sludge. At the last period of laboratory operation, the sludge remained in the reactor stratified with the larger ones settling down in the lower part of reactor and the smaller ones expanded or suspended in the upper part of sludge-bed due to the mixing smaller ones expanded or suspended in the upper part of sludge-bed due to the increasing of upflow velocity.

### 4.4.3 Nutrient Removal

#### 1) Nitrogen Removal

**a) Nitrogen removal versus hydraulic retention time (HRT):** Nitrogen constituents in terms of nitrate nitrogen ($NO_3^-$-N) and organic nitrogen were removed with the efficiencies of 54 – 78 and 65 – 83%, respectively for 12-h – 24-h hydraulic retention time as shown in Figure 4.9. The maximum removal of nitrate nitrogen was achieved at 20-h hydraulic retention time whereas the removal of organic nitrogen was found at 16-h HRT**.**

At 20-h and 24-h HRT, the removal efficiencies of nitrate nitrogen were not different, whereas at 16-h and 12-h HRT, the removal efficiencies were decreased significantly. In case of organic nitrogen, the removal efficiencies for 16-h to 24-h of HRT were similar and sharply decreased at 12-h HRT. This result indicated that HRT would not affect to the removal efficiency of organic nitrogen and nitrate nitrogen for HRT of 16-h to 24-h.

**b) Nitrogen removal versus nitrogen loading rate (NLR):** The removals of nitrate nitrogen were in the ranges of 54 to 78% while 64 to 83% were found in the removal of organic nitrogen as presented in Figure 4.10. It can be

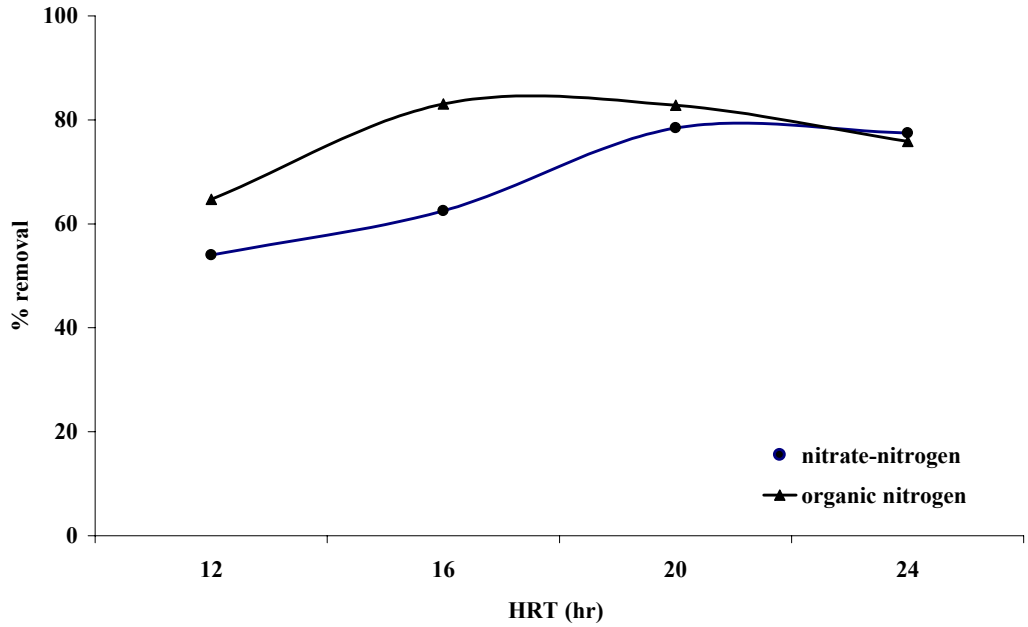



**Figure 4.9** The removal efficiency of nitrate nitrogen and organic nitrogen at various hydraulic retention times.

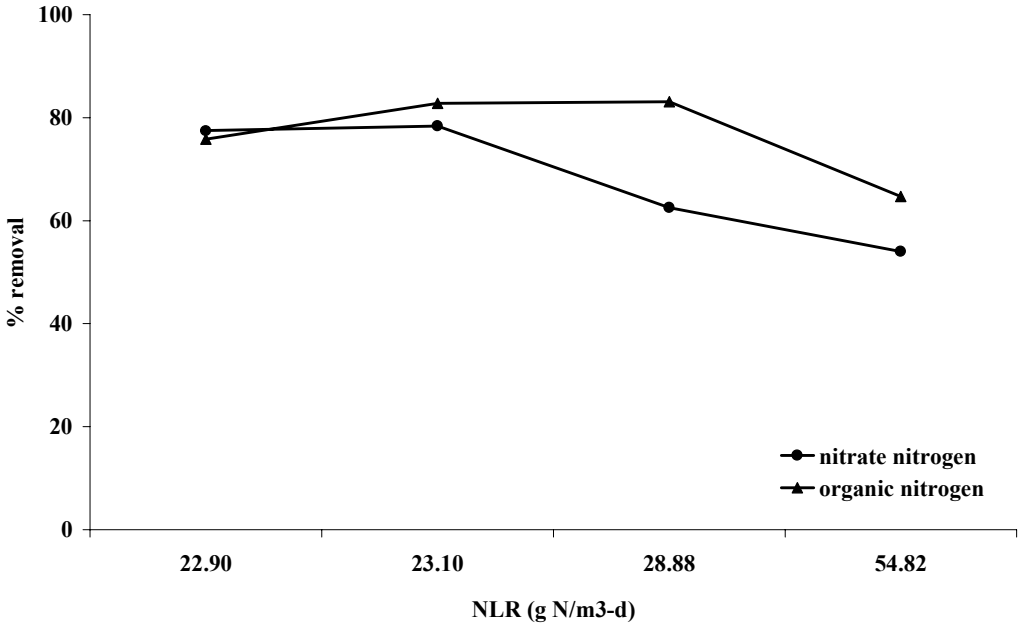



**Figure 4.10** Removal efficiencies of nitrate nitrogen and organic nitrogen at various nitrogen loading rates (NLR).

clearly observed that the excellent organic nitrogen removed (greater than 70%) was found for nitrogen loading rate of 22.9 to 28.88 g NLR/$m^3$-d. While the good removal of nitrate nitrogen were in the ranges of 22.9 to 23.1 g NLR/$m^3$-d, the removal efficiencies of both nitrate and organic nitrogen were sharply decreased for NLR exceeded 23.1 and 28.88 g NLR/$m^3$-d, respectively. The result of this study indicated that the removal efficiencies of nitrate and organic nitrogen were related to the nitrogen loading rate and short retention time (less than 12-h).

**c) Nitrogen extraction:** Most nitrogen in dairy wastewater used for this study was in the form of organic nitrogen, and some nitrate nitrogen ($NO_3^-$–N) as well. The proportions of organic nitrogen to total nitrogen were in the ranges of 95-99% with 97% of average values, and the remaining was inorganic nitrogen compound such as nitrate nitrogen as illustrated in Figure 4.11. The ratios of nitrate nitrogen to total nitrogen in influent were small fractions with only 0.4-4.6%. The ammonia nitrogen in the influent was not found in this study for all HRTs, whereas it could be monitored at the effluent with the percentage ranges of 51-83% and 73% of mean value. Almost of organic nitrogen in dairy wastewater were converted to ammonia nitrogen due to bacterial composition and hydrolysis as illustrated in reaction (4-1) and later assimilate to organic nitrogen in bacterial cells. Moreover, organic nitrogen in bacterial cells was also converted to ammonia nitrogen according to the death and hydrolysis of cell. Regarding to nitrate nitrogen, it was reduced to nitrite nitrogen form and later assimilatively reduced to ammonia nitrogen as so-called ammonification by the action of bacteria under anaerobic conditions as shown in chemical reaction (4-2). The nitrogen extraction to be nitrogen constituents can be shown in simplified mass balance diagram for each HRT in Figure 4.12.

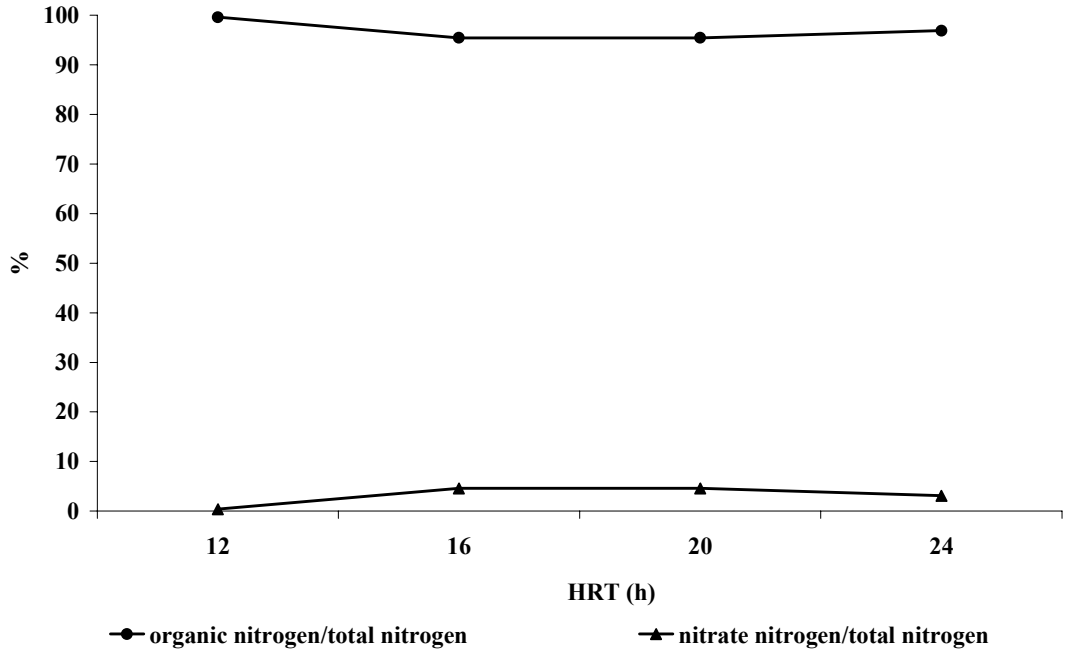



**Figure 4.11** The proportion of organic nitrogen and nitrate nitrogen to total nitrogen in influent for each hydraulic retention times.

Dairy wastewater (organic nitrogen) + bacteria $\rightarrow$ $NH_3$ (4-1)

Similarity of nitrate-nitrogen, it was converted to ammonia through assimilative reduction for use in cell synthesis as follows:

$$NO_3^- \xrightarrow{\text{reduction}} NO_2^- \xrightarrow{\text{assimilative reduction}} NH_3 \qquad (4\text{-}2)$$

Ye and Thomas (2001) indicated that anaerobic metabolism of *Bacillus subtilus* played a role of dissimilatory or assimilatory nitrate reduction to ammonia via nitrite similar to chemical reaction 4-5. Assimilatory reduction of nitrate to ammonia ammonia via nitrite enabled microbes to use nitrate as the nitrogen source. *Bacillus subtilus* was capable of using nitrate and nitrite as the alternative electron acceptors to support anaerobic growth. Other researchers also indicated that under anaerobic environment, nitrogen consumed was assimilated into the cell for

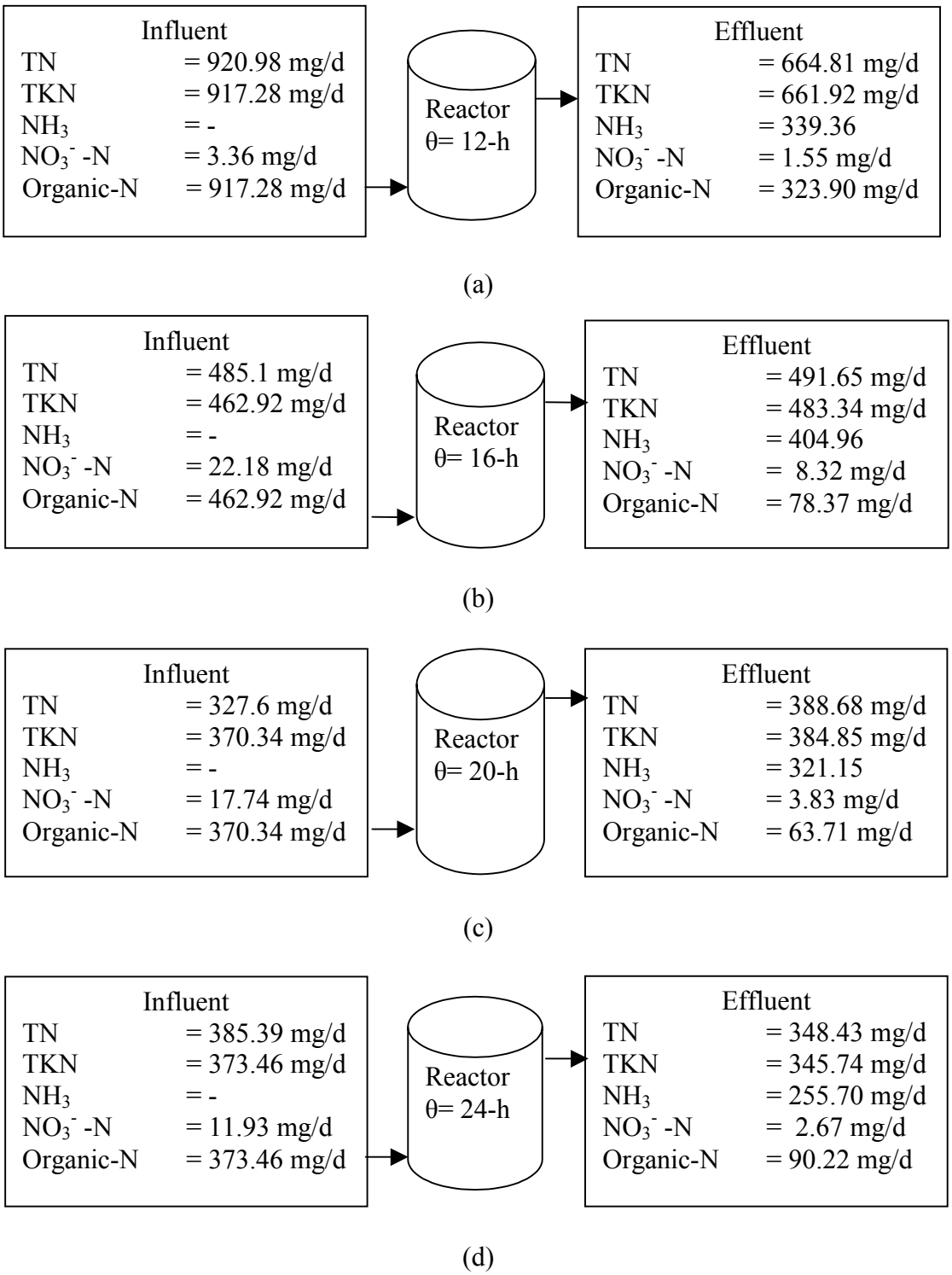



**Figure 4.12** Mass balance diagram of UASB process operations for nitrogen extraction in each hydraulic retention times (mg/d).

biosynthesis (Panswad et al., 2003).

**2) Phosphorus Removal**

**a) Phosphorus removal versus HRT: The concentrations of** organic phosphorus in influent and effluent were in the ranges of 0.15-0.6 mg/L and 0.04-0.14 mg/L, respectively. The maximum removal of organic phosphorus was observed at 12-h hydraulic retention time with 95% efficiency whereas the minimum was observed at 20-h with 68% of removal efficiency as shown Figure 4.13. At 16-h to 24-h HRT, the removal efficiencies of organic phosphorus were not significantly different (68-73%).

**b) Phosphorus removal versus phosphorus loading rate (PLR):** The pattern of organic phosphorus removal in this study was quite different from other parameters as illustrated in Figure 4.14. The removal efficiencies were fluctuated for each phosphorus loading rate, the maximum removal (95%) was observed at phosphorus loading rate (PLR) 3.6 g PLR/$m^3$-d and minimum removal (68%) was found at 1.29 g PLR/$m^3$-d. The removal efficiencies of organic phosphorus were not different for the phosphorus loading rate ranging from 0.49 to 1.6 g PLR/$m^3$-d. The results of this may be induced from the consumption of phosphorus by microorganism and some were released from microorganism itself under anaerobic condition. In addition, the results obtained from this study could not clearly indicate whether, after 3.6 g PLR/$m^3$-d, the removal efficiency would be increased or decreased.

**c) Phosphorus extraction:** The dairy wastewater contained ortho-phosphate and organic phosphorus with the ranges of 59-69% or average value of 63% and 31-41% or 37% of mean value, respectively. Regarding to the treated wastewater, major content was ortho phosphate with the average value of 91% whereas the organic

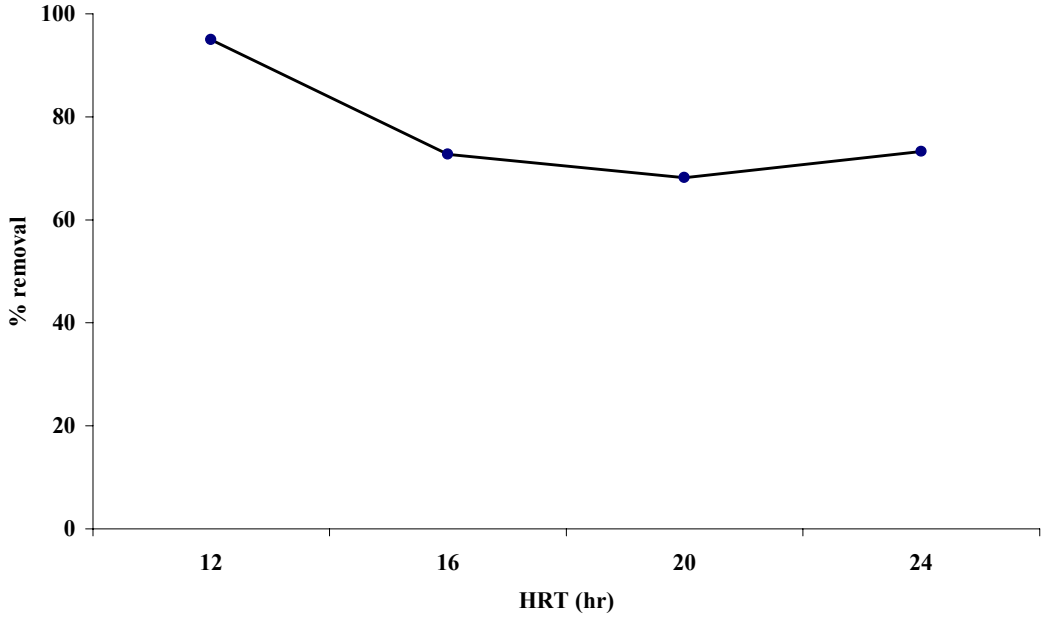



**Figure 4.13** The removal efficiency of organic phosphorus for each hydraulic retention times.

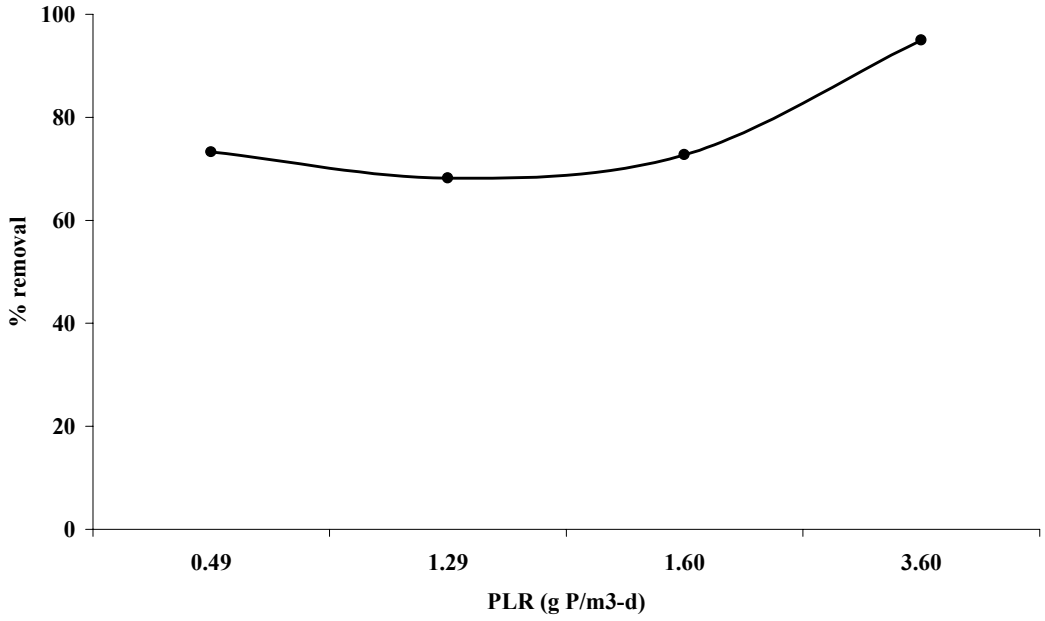


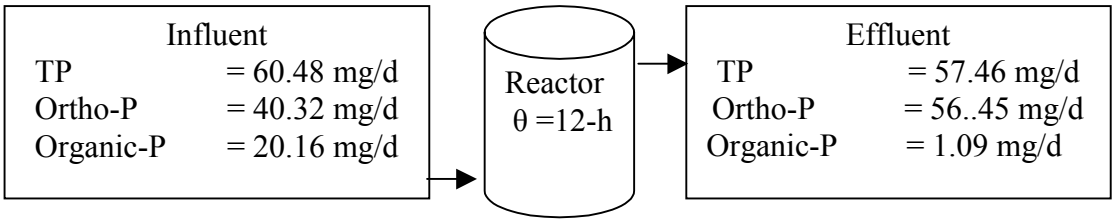

**Figure 4.14** The removal efficiencies of organic phosphorus at various phosphorus loading rates (PLR).

phosphorus was found only 9% of average value. It could be concluded that organic phosphorus was converted to ortho-phosphate in acidogenic step and some organic phosphorus were used by microorganisms for cell synthesis and energy transport but also stored for subsequent use (Metcalf & Eddy, 1991). The phosphorus utilization and release can be illustrated in schematic mass balance diagram for each hydraulic retention times in Figure 4.15. Biological phosphorus removal is a complex process that is dependent on the growth of specialized phosphate accumulating organisms (PAOs), which store phosphorus as polyphosphate (Poly-P). Under anaerobic conditions, PAOs do not grow, but store acetic acid as PHB (Poly-β-hydroxybutyrate) through the cleavage of Poly-P with the associated eslease of soluble phosphorus (Grady et al., 1999). Therefore, the concentration of ortho-P in effluent were higher than influent concentration for all HRTs of this study. In case of total phosphorus, it can be observed that at 12-h to 20-h HRT, the total phosphorus concentrations of influent were higher than effluent, meaning that some amount of phosphorus was used by microorganisms. Whereas at 24-h HRT, total phosphorus concentration of influent was less than effluent concentration. It revealed that some phosphorus might be released from miroorganisms similar to the Mino model, biological phosphorus removal is a complex process that is dependent on the growth of specialized phosphate accumulating organisms (PAOs). This could be the effect of uptake and release of phosphorus by PAOs.

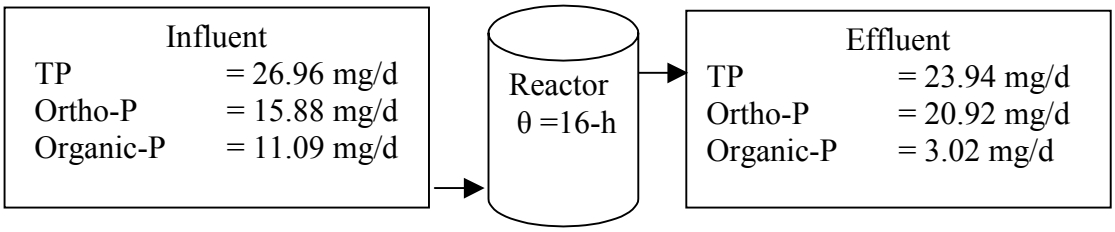
## 4.5 Granule Size

### 4.5.1 Granule Size Determination

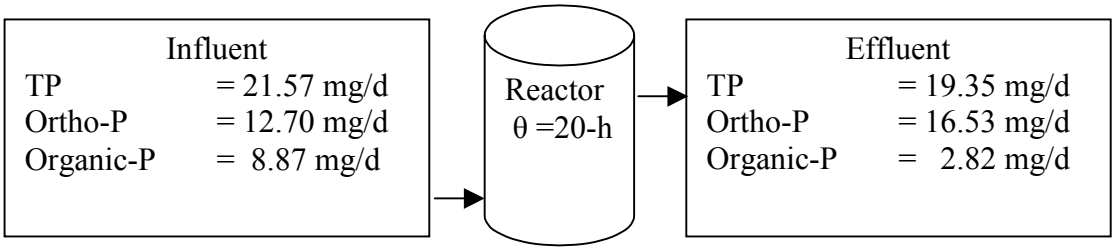
Anaerobic granule formation was started from the microbial adhesion



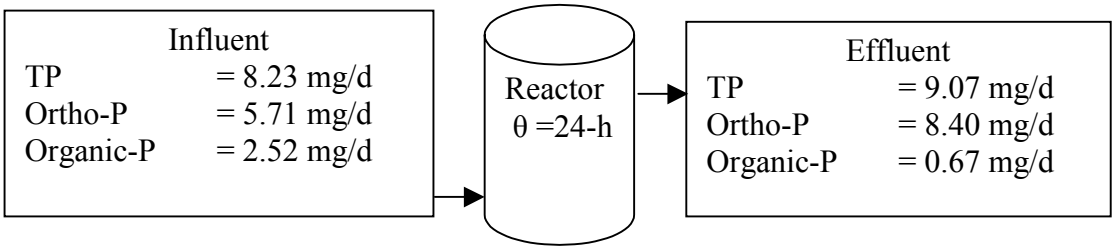

(a)




(b)




(c)




(d)

**Figure 4.15** Schematic mass balance diagram of UASB process operation for phosphorus extraction in each hydraulic retention time (mg/d).

or self-immobilization that could be defined in terms of the energy involved in the interaction of bacterium-to-bacterium or bacterium-to-solid surface. When one bacterium approaches another, the interaction between them includes repulsive electrostatic force, attractive van de waals force, and repulsive hydration interaction. In this study, the formed granular sludge obtained from the beverage plant (Pepsi Wastewater Treatment Plant), Nonthaburi province, was initially used in the reactor. After reaching the steady state condition of each HRT or flow rate, the size of granule was classified by sieve analysis method and the results are illustrated in Table 4.4 and Figure 4.16. It was found that the average granule sizes ranged from 1.35 to 1.71 mm for the flow rate of 16.8 to 33.4 L/d or equivalent to upflow velocity of 0.045 to 0.090 m/hr, respectively. The average value of granule size for four flow rates were 1.53 mm. The major component (47%) of granule size was 1.19 mm for all upflow velocities. The shape of size distribution can be seen as normal distribution for the whole flow rates as illustrated in Figure 4.17 or can be presented in the form of accumulated percentage in Figure 4.18.

### 4.5.2 Relationships Between Granule Size and Flow Rate

The Z-test was employed to determine the diffrence of granule size for each flow rate, it revealed that that the granule sizes were not significantly different as shown in appendix C($\alpha$=0.05). Similar to the study undertaken by Boonyakitsom but et al. (2002), the re-granulation of anaerobic sludge in UASB were not significant different for the ranges of upflow velocity 0.02 to 0.5 m/hr. The charactristics of granule ramained in reactor for each size are presented in Figure 4.19 and Figure 4.20.

**Table 4.4** The percentage of granule size for various influent flowrates.

| Average granule size (mm) | The percentage of granule size for various flow rate | | | |
|---|---|---|---|---|
| | 16.8 L/day | 20.16 L/day | 25.2 L/day | 33.4 L/day |
| 2.36 | 26.68 | 34.66 | 27.06 | 10.74 |
| 2.00 | 5.38 | 19.20 | 11.18 | 15.14 |
| 1.19 | 63.81 | 35.28 | 44.58 | 42.60 |
| 1.00 | 2.96 | 6.82 | 12.53 | 24.81 |
| 0.84 | 0.79 | 2.18 | 3.37 | 5.19 |
| 0.59 | 0.31 | 0.90 | 0.90 | 1.08 |
| 0.42 | 0.03 | 0.37 | 0.23 | 0.26 |
| 0.25 | 0.02 | 0.59 | 0.15 | 0.18 |
| Mean(mm) | 1.52 | 1.71 | 1.54 | 1.35 |

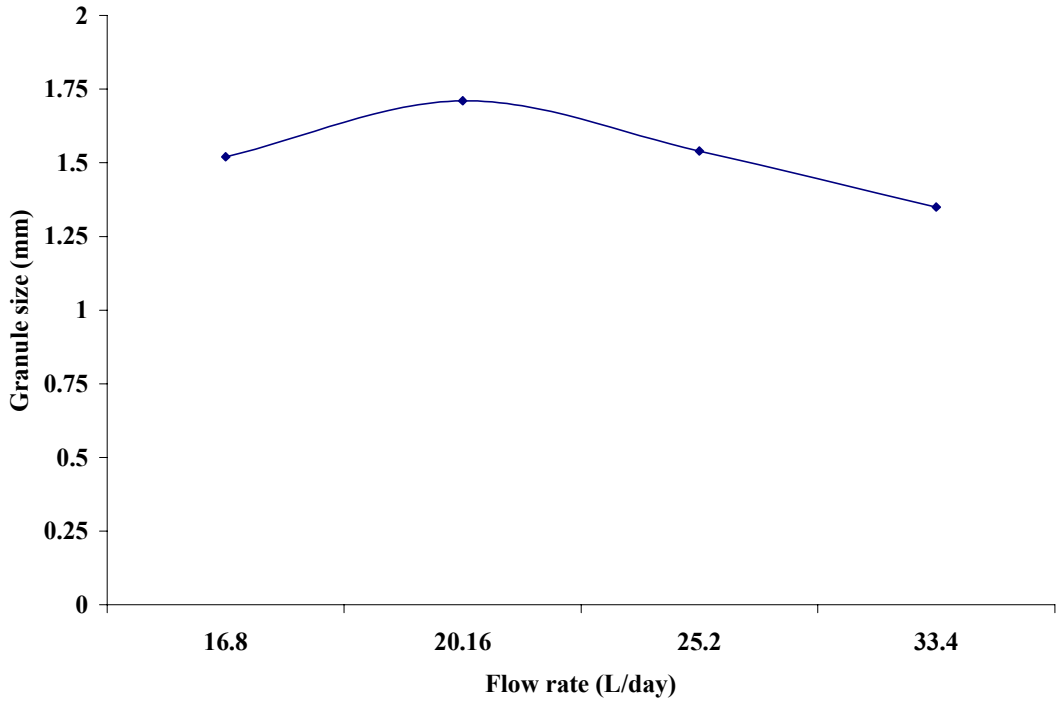



**Figure 4.16** The relationship of granule size (mm.) and influent flow rate (L/day).

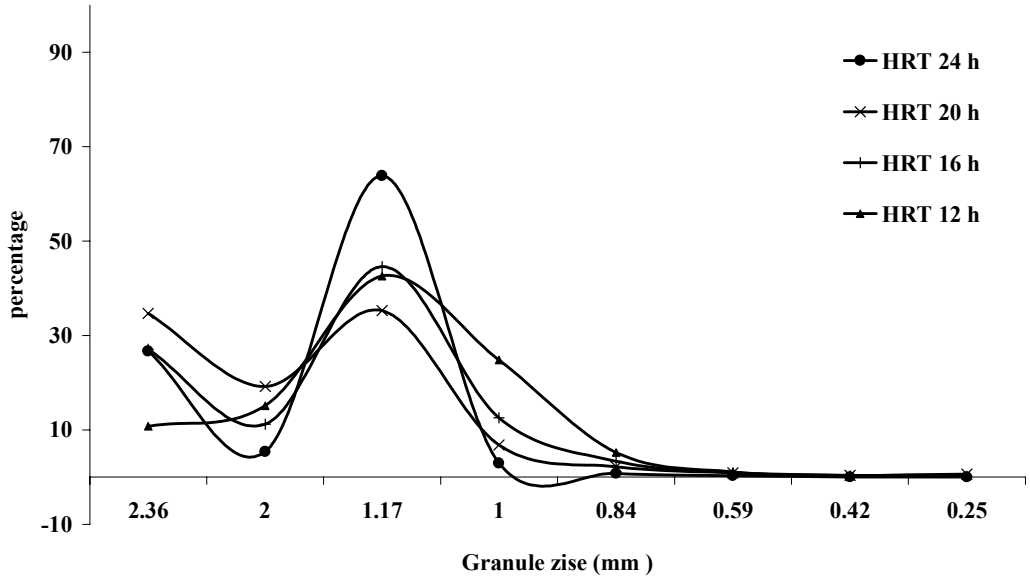



**Figure 4.17** The granule size distribution for various hydraulic retention times or influent flow rate.

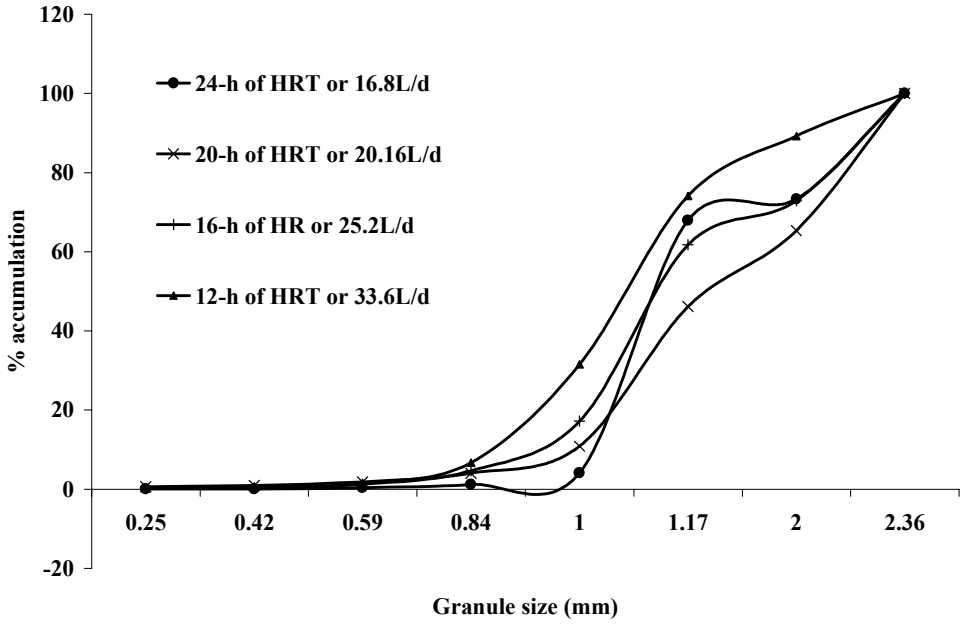



**Figure 4.18** The percentage of accumulation of granule size.

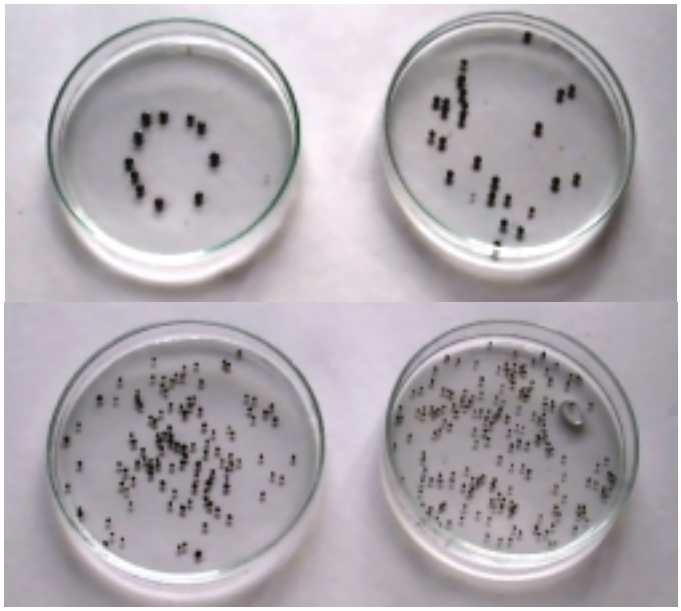



**Figure 4.19** The characteristics of granule remained in reactor for the granule size 1.00 to 2.36 mm.

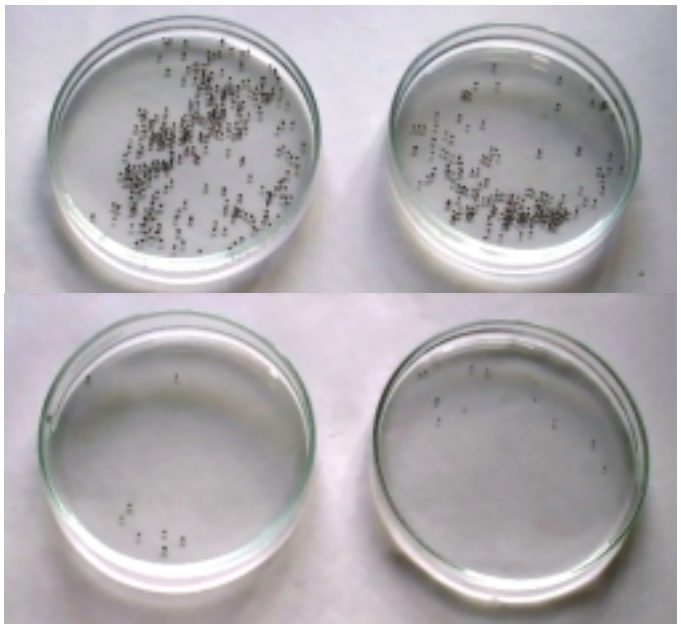



**Figure 4.20** The characteristics of granule remained in reactor for the granule size 0.250 mm. to 0.841 mm.

## 4.6 Biogas Production and Composition

The daily biogas production and gram COD removed at the steady state were analyzed, it can be seen that 1 kg COD used can produce 552 liters (L) of biogas. The relationship of g COD used and mL of biogas produced is linearized below. It is illustrated in Figure 4.21 with the details of calculation shown in Appendix D.

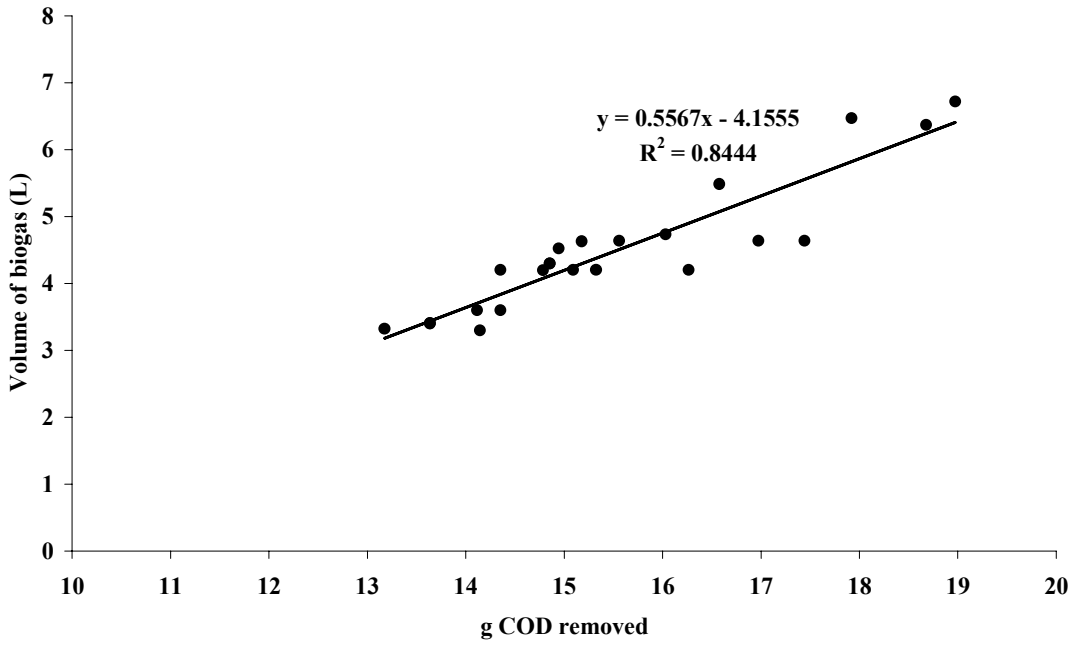



**Figure 4.21** The relation of g COD removed and volume of biogas production.

$$Y = 556.65X - 4155.5 \quad (4\text{-}3)$$

$$R^2 = 0.84$$

Where

Y = biogas production (mL)

X = g COD used

The negative value of above equation indicated that minimum of 7.4 g COD

used would produce the milliliter of biogas. The volumetric biogas production rate increased kg slightly linearly with the COD loading rate, until reaching a maximum 6.8 L/d at OLR of 1.28 kg COD/$m^3$-d as illustrated in Figure 4.22. It indicated that the increase of flow rate from 16.8 to 25.2 L/d, caused the increasing rate of biogas production from 4 L/d to 6.8 L/d. The results of the increase of upflow velocity not only caused the increase of biogas formation but also the increase of suspending of smaller size of sludge to the upper part. This study agreed with the results of Ramasamy and Abbasi (2000), they indicated that the biogas yield was increasing when HRT was brought down.

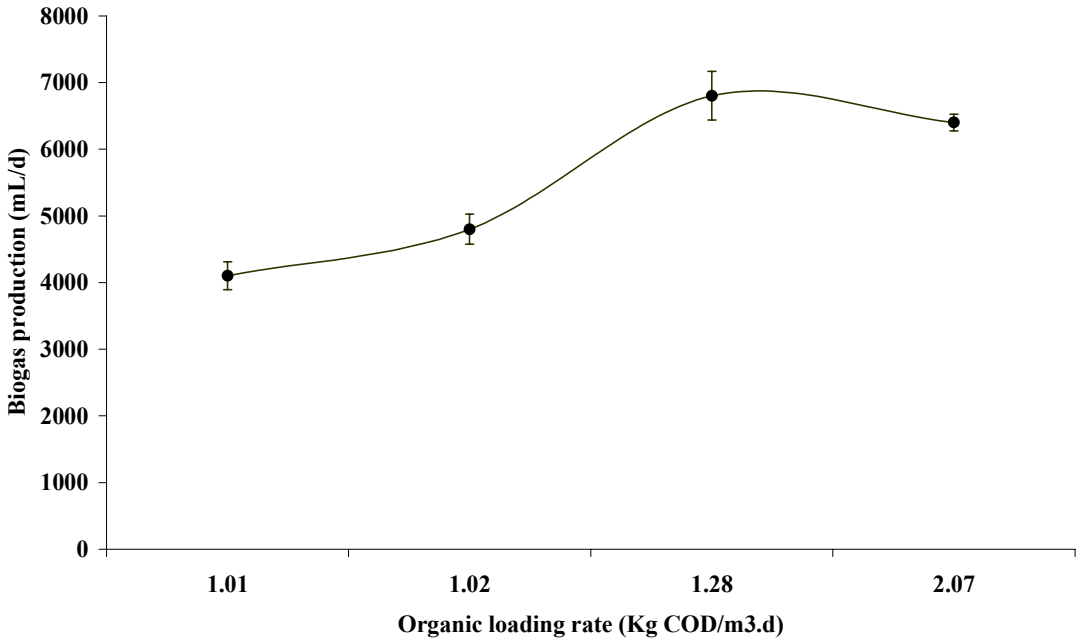



**Figure 4.22** Biogas production rate for each organic loading rates.

In case of biogas composition, the methane was found in the ranges of 60-80% or average value of 68%. As mentioned above, 552 L of biogas was generated from 1

kg COD used meaning 375 L of methane was produced from that amount of COD utilized. This value is slightly inferior to the stoichiometric theoretical of 0.35 $m^3$ $CH_4$/kg COD and similar to the result studied by Perez et al. (2001), 0.33 $m^3$ $CH_4$/kg COD. The result studied by Yan and Tay (1996) also had similar methane, yield of 300 L $CH_4$ per kilogram of COD removed, was produced based on the continuous operation by using UASB reactor treating brewery wastewater. Whereas yield of methane studied by Rodrignez-Martinez et al. (2002) in the treatment of slaughterhouse wastewater in UASB reactor had methane content slightly higher than Yan and Tay with the valume of 349 mL per 1 gram of COD utilized. The study, reported by Rajeshwari et al. (2000), methane composition with pH control for the treatment of cheese whey wastewater by anaerobic degradation ranged from 70.8 to 71%.

## 4.7 Acid Distributions

The organic acid distribution of effluent observed in this laboratory-scale reactor were lactic, acetic, butyric and propionate acid. In case of influent, they were not analysed because the dairy wastewater was just prepared and the fermentation had not occurred. Therefore, the acid distribution in influent was negligible. In an anaerobic wastewater treatment system, organics in dairy wastewater were converted to amino acids and simple molecule of sugars in hydrolytic phase and would be degraded by fermentative reaction to be volatile acids (propionic acid, butyric acid, lactic acid and acetic acid) as so-called acidogenesis. As illustrated in Table 4.5, the variation of HRT had little effect on the effluent concentration of propionic acid, acetic acid and butyric acid. This result indicated that the effect of HRT of 12-h to 24-

h was insignificant on the degradation of dairy wastewater. Compared with the similar results reported by Fang and Yu (2000), the degradations of proteins were in the ranges of 80 to 86% for the HRT of 12-h to 24-h, respectively. The variation of HRT had little effect on the effluent lactate concentration by using 2.8-L upflow anaerobic reactor treating dairy wastewater.

**Table 4.5** The acid concentration in effluent in ppm unit at various hydraulic retention times.

| Acid (ppm) | HRT (h) | | | |
|---|---|---|---|---|
| | 12 | 16 | 20 | 24 |
| Propionic | 7.54 | 6.95 | 5.1 | 7.77 |
| Lactic | 45.24 | 27.64 | 25.35 | 8.74 |
| Acetic | 8.06 | 7.79 | 7.31 | 10.32 |
| Butyric | 6.02 | 5.59 | 5.14 | 6.96 |

Since the proportion of fresh milk and sugar used for this study were 95 and 5%,respectively, then the major contents were amino acids and simple sugars in hydrolytic phase. In acidogeneic phase, the amino acids and sugars were degraded by fermentative reactions in which organic compound serve as both electron donors and acceptors.

In this study, the organic compound in anaerobic condition was assumed to be converted to organic acids and later to acetic acid, and finally to methane and carbon dioxide gas as shown in the schematic diagram in Figure 4.23. The portion of conversion from organic compound to organic acids of propionate, butyrate and lactate obtained from this study were 17.3, 15 and 67.7%, respectively. The organic

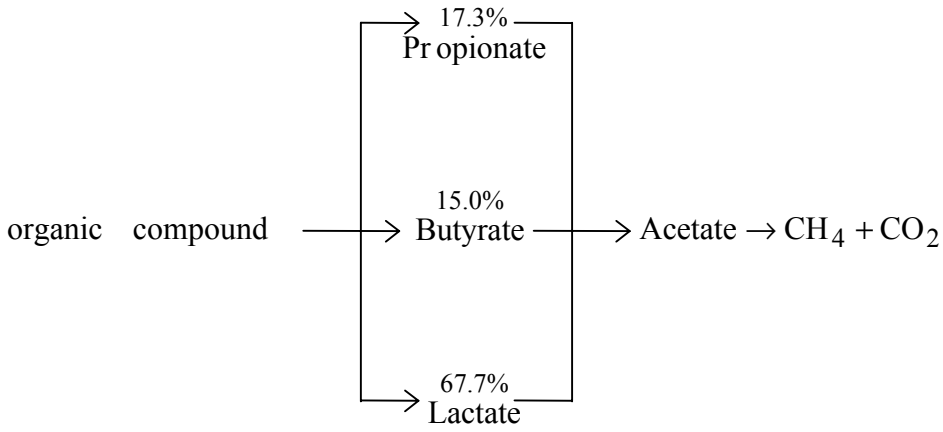



**Figure 4.23** The conversion of organic compounds in anaerobic condition.

acids were almostly converted to acetic acid as the following reactions.

Lactic acid

$$2CH_3CHOHCOOH \rightarrow 3CH_3COOH \quad (4\text{-}4)$$

Propionic acid

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2 \quad (4\text{-}5)$$

Butyric acid

$$2CH_3CH_2CH_2COOH + 8H_2O \rightarrow 2CH_3COOH + 4CO_2 + 12H_2 \quad (4\text{-}6)$$

Acetic acid

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (4\text{-}7)$$

Regarding to the material balance of organic acid equivalent to COD concentration, it can be estimated as theoretical value for each acid and HRT by the following equations.

Lactic acid

$$\underset{90}{CH_3CHOHCOOH} + \underset{96}{3O_2} \rightarrow 3CO_2 + 3H_2O \quad (4\text{-}8)$$

Propionic acid

$$\underset{74}{CH_3CH_2COOH} + \left(\tfrac{7}{2}\underset{112}{O_2}\right) \rightarrow 3CO_2 + 3H_2O \quad (4\text{-}9)$$

Butyric acid

$$\underset{88}{CH_3CH_2CH_2COOH} + 5\underset{160}{O_2} \rightarrow 4CO_2 + 4H_2O \quad (4\text{-}10)$$

Acetic acid

$$\underset{60}{CH_3COOH} + 2\underset{64}{O_2} \rightarrow 2CO_2 + 2H_2O \quad (4\text{-}11)$$

The followings are the example of COD equivalent calculation by estimation from acid distribution.

Lactic acid at 12-h HRT

$$\text{COD equivalent} = \left(\frac{90}{60}\right) \times \text{Lactic concentration}$$

$$= \left(\frac{90}{60}\right) \times 60.53$$

$$= 64.57 \text{ mg/L}$$

Other acid types and other HRTs were estimated by the similar manner. In theoretical, the summation of COD of each acid should be equal to COD effluent as following equation.

$$COD_{effluent} = \text{Total} \quad COD_{acid} \quad (4\text{-}12)$$

$$\text{Total} \quad COD_{acid} = COD_{lactic} + COD_{propionic} + COD_{acetic} + COD_{butyric} \quad (4\text{-}13)$$

The estimated COD of each acid and HRT are presented in Table 4.6. In this study, total $COD_{acid}$ for all HRTs were lower than $COD_{effluent}$. The reason of this may be caused by the oxidation process of some organic compounds and other compounds

during digestion and they were reacted with dichromate, then COD values calculated from acid concentration were lower than $COD_{effluent}$.

## 4.8 Determination of Kinetic Coefficients

The parameters of Y, k, and $K_s$ must be estimated for use in the biological wastewater treatment model. To determine the above coefficients values, the results obtained from this study were used.

**Table 4.6** The calculated COD obtained from acid concentration and monitored $COD_{effluent}$ for each HRT.

| Acid (ppm) | HRT (h) | | | |
|---|---|---|---|---|
| | 12 | 16 | 20 | 24 |
| Propionic | 11.41 | 10.52 | 7.72 | 11.76 |
| Lactic | 48.26 | 29.48 | 27.04 | 9.32 |
| Acetic | 8.60 | 8.31 | 7.80 | 11.01 |
| Butyric | 10.95 | 10.16 | 9.35 | 12.65 |
| Total $COD_{acid}$ | 79.21 | 58.48 | 51.90 | 44.75 |
| $COD_{effluent}$ | 351.10 | 84.16 | 65.7 | 106.74 |

In this study, the four different HRTs were used to determine the coefficient values. During steady-state condition, the biomass was not changed then it was assumed as constant for kinetics coefficient determination. The collected data such as Q, $S_o$, S, X for each HRT in terms of mean value were used to determine kineticscoefficients by using Monod and Levenspiel models. The details of calculation are shown in appendix E. The values of kinetic parameters (k and $K_s$) were estimated from Figure 4.24, the linear equation as follow.

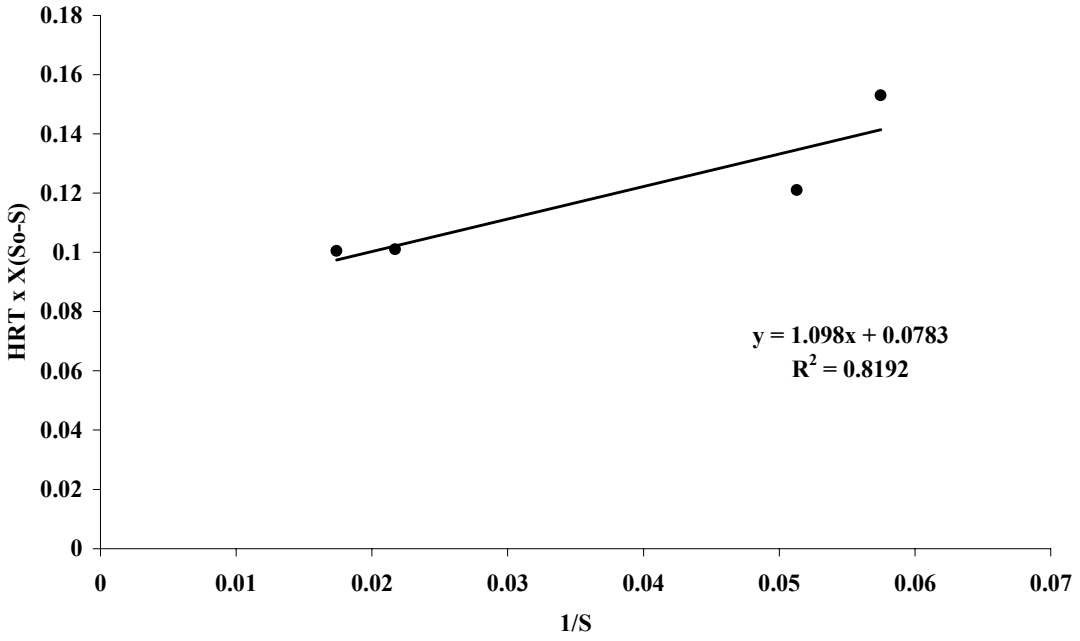



**Figure 4.24** The relationship of $\frac{\theta X}{S_0 - S}$ versus $\frac{1}{S}$ used for determination of k and $K_s$ values.

$$HRT \times X(S_o - S) = 1.098\left(\frac{1}{S}\right) + 0.0783 \qquad (4\text{-}14)$$

Whereas Y was calculated from Figure 4.25, the related equation in forms of linear is shown below.

$$\frac{1}{HRT} = \frac{0.19(S_0 - S)}{HRT \times X} \qquad (4\text{-}15)$$

The relationship between specific growth rate (μ) and substrate (S) was illustrated in Figure 4.26. Comparing to kinetic parameters of anaerobic investigated by other researchers (Table 4.7), the parameters obtained from this study were in the ranges of these values. The value of $K_s$ gained in this study was slightly lower than that of other researchers but higher than that of Hu et al. (2002).

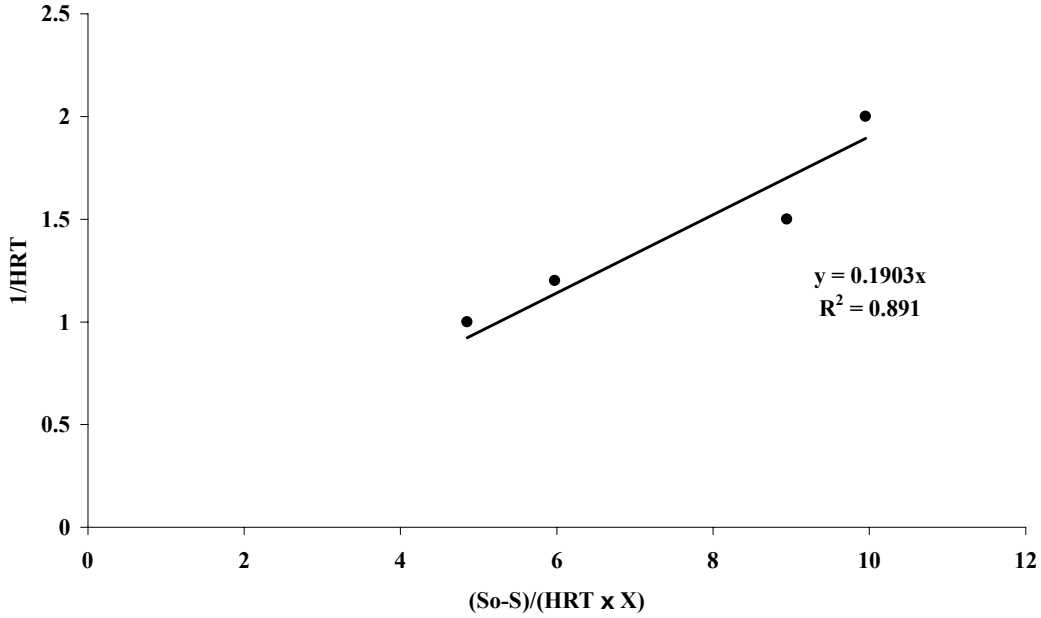



**Figure 4.25** The relationship of $\frac{1}{\theta_c}$ versus $\frac{S_0 - S}{\theta X}$ used for determination of $k_d$ and Y.

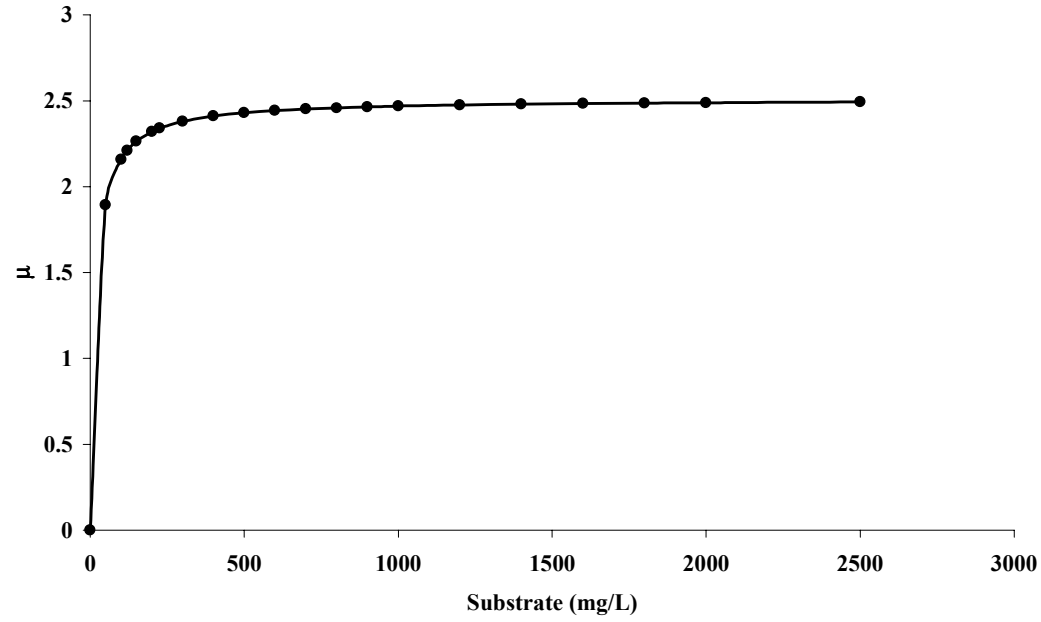



**Figure 4.26** The relationship of specific growth rate ($\mu$) and substrate utilization (S).

**Table 4.7** Typical values of kinetics constants indicated by various researchers compared with this study.

| Component | Y, g VSS/g COD | k, ($d^{-1}$) | $K_s$, (mg/L) | $k_d$, ($d^{-1}$) |
|---|---|---|---|---|
| **Jeyaseelan (1997)** | | | | |
| *Acid phase* | 0.14 | 90 | 450 | 6.1 |
| Carbohydrates | 0.128 | 20 | 500 | 0.014 |
| Proteins/ Lipids | 0.10 | 12 | 850 | - |
| *Methane phase* | | | | |
| Acetic acid/ acetate | 0.03 | 6 | 400 | 0.037 |
| **Nopharatana et al. (2003)** | 0.204 | 11.77 | 500 | 0.048 |
| Acidogenic bacteria | 0.0232 | 6 | 360 | 0.101 |
| Acetoclastic methane bacteria | 0.01588 | 8.75 | 30 | 0.048 |
| Hydrogen utilizing methane bacteria | 0.2116 | 3.71 | 0.403 | 0.013 |
| Hu et al. Ice-cream wastewater | | | | |
| ***This study*** | ***0.19*** | ***13*** | ***14.73*** | **-** |

Regarding to the reaction rates of constant for this UASB reactor, it can be determined by each step of digestion as illustrated in the equation below.

$$\text{Organic compound} \xrightarrow{r_1} \text{Organic acid} \xrightarrow{r_2} \text{Acetic acid} \xrightarrow{r_3} CH_4 \qquad (4\text{-}16)$$

(A) (B) (C) (D)

Hydrolysis Acidogenesis Methanogenesis

The first reaction ($r_1$) was assumed as hydrolysis phase of anerobic wastewater treatment process, the second reaction of $r_2$ was acidogenesis phase and the last reaction was methanogenesis phase.

The material balance was performed with the following assumptions.

(1) Only major steps of hydrolysis, acidogenesis and methanogenesis are significant. The proposed mechanism is given in equation (4-16).

(2) Microbial growth is negligible in stoichiometry.

(3) The model is based on the unit of COD due to the complexity and variability of substrate concentrations.

(4) Methane is undissolved in the liquid.

The reaction rate of organic compound to organic acid was found to be the maximum rate for all HRTs as shown in Table 4.8, whereas the minimum reaction rate was observed at reaction rate ($r_3$) of acetic acid conversion to methane gas. The average values of reaction rate of 4 HRTs were 0.448, 0.019 and 0.004 mg COD/L-h for $r_1$, $r_2$ and $r_3$, respectively. It could be said that eventhough the flow rate was increased, the reaction rates were not changed as shown in Figure 4.27. From overall process, it revealed that the reaction rate ($r_3$) was determined as the limiting rate of reaction of dairy wastewater treatment process as only approximately 1% of total reaction rate. On the other hand, it can be said that the process of converting acetic acid to methane gas or methanogenesis phase, needed a long period.

**Table 4.8** The reaction rate of each step and HRT.

| HRT (h) | (A)→(B) $r_1$, (mg COD/L-h) | (B)→(C) $r_2$ (mg COD/L-h) | (C)→(D) $r_3$ (mg COD/L-h) |
|---|---|---|---|
| 12 | 0.446 | 0.029 | 0.004 |
| 16 | 0.446 | 0.023 | 0.005 |
| 20 | 0.449 | 0.020 | 0.004 |
| 24 | 0.451 | 0.011 | 0.005 |
| Average | 0.451 | 0.019 | 0.0045 |

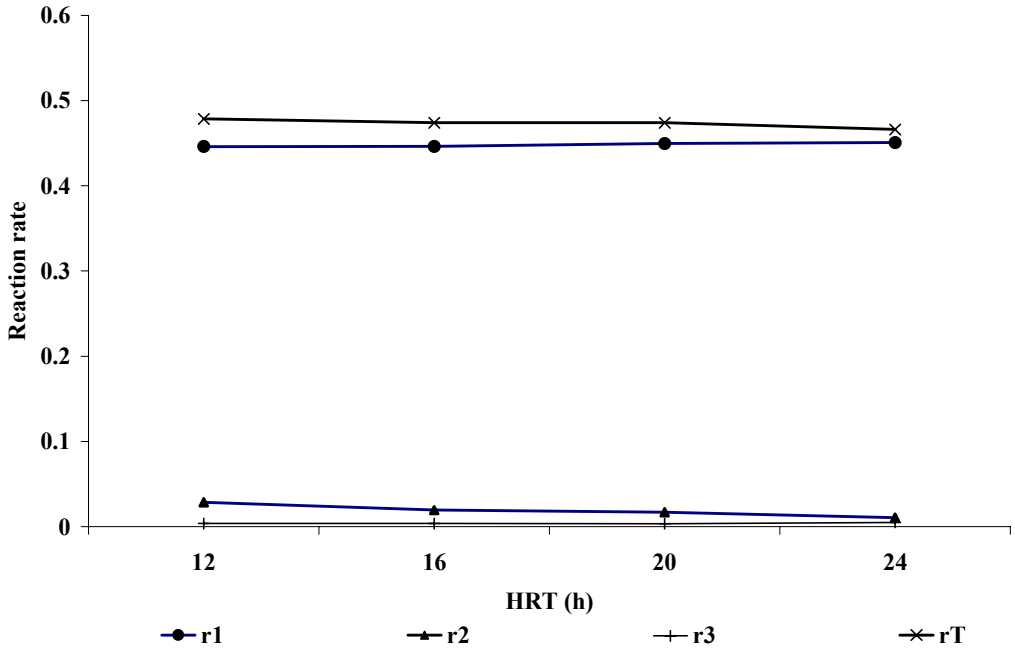



**Figure 4.27** The reaction rate of dairy wastewater for each hydraulic retention times.

Regarding to total reaction rate, it was calculated by the following equation.

$$\text{Total reaction rate (rT)} = r_1 + r_2 + r_3 \quad (4\text{-}17)$$

The $r_T$ and proportion of each reaction rate to total reaction rate were shown in Table 4.9. The proportion of $r_1$ was the maximum rate with the average value of 0.95 mg COD/L-h whereas the minimum was $r_3$ of 0.009 mg COD/L-h.

## 4.9 Model Formulation

In general, the model can be applied for many purposes. Costanza and Gottlieb (2001) considered three uses of model: understanding, assessing, and optimizing. Models can be used to gain a conceptual picture of how a system of interest might work. In many cases, these types of models are generated before any field or laboratory studies have been conducted, and their main purpose is to examine what

**Table 4.9** The proportion of each reaction rate to total reaction rate for each HRT.

| HRT (h) | $r_T$ (mg COD/L-h) | Proportion of reation rate in each step to $r_T$ | | |
|---|---|---|---|---|
| | | $r_1$ (mg COD/L-h) | $r_2$ (mg COD/L-h) | $r_3$ (mg COD/L-h) |
| 12 | 0.479 | 0.93 | 0.06 | 0.008 |
| 16 | 0.474 | 0.94 | 0.04 | 0.008 |
| 20 | 0.474 | 0.95 | 0.04 | 0.008 |
| 24 | 0.466 | 0.97 | 0.02 | 0.011 |
| Average | 0.473 | 0.95 | 0.04 | 0.009 |

features are the most critical in determining system behavior. In this study, a model is carried out accompanying with the experiment, with the purpose of understanding the UASB process and will be used as a tool in investigation of the system such as methane production.

There are a large variety of software tools currently available for simulation modeling, STELLA was one of the first dynamic modeling systems to achieve broad recognition and use.

### 4.9.1 Model Equation

The Monod model of substrate utilization and microorganism were employed for this study, kinetics parameters obtained from this study as indicated in section 4.4 were used as input. Mass balance equations for microorganism and limiting substrate in a continuous flow system can be estimated by equating accumulation against the increases and decreases occurring in an infinitely short time interval as follows.

| Rate of accumulation of microorganism within the system boundary | = | Rate of flow of microorganism into the system boundary | - | Rate of flow of microorganism out of the system boundary | + | Net growth of microorganism within the system boundary |
|---|---|---|---|---|---|---|

This can be written as :

Accumulation = Inflow - Outflow + Net growth

$$V\left(\frac{dX}{dt}\right) = QX_0 - QX + \mu X \qquad (4\text{-}18)$$

**a) Microorganisms:**

In this study, a UASB without biomass recycle, the rate of biomass change in the reactor can be expressed as equation below.

$$\frac{dX}{dt} = \left(\frac{Q}{V}\right)X_0 - \left(\frac{Q}{V}\right)X + \mu X \qquad (4\text{-}19)$$

Where

Q = the flow rate, L/day

V = the volume of the reactor, L

$X_0$ = the concentration of biomass in the feed, g VSS/L

X = the concentration of biomass in the reactor, g VSS/L

$\mu$ = specific growth rate, $day^{-1}$

At steady-state, it was assumed that the concentration of biomass in the influent was neglected, then $\frac{dX}{dt} = 0$. The HRT($\theta$) was defined as the volume of reactor divided by flow rate of influent $\left(\theta = \frac{V}{Q}\right)$. The biomass at the steady-state

conditions can be estimated by the following equation.

$$X = Y(S_0 - S) \tag{4-20}$$

**b) Substrate**

The rate of change in substrate concentration in the reactor could be expressed as:

$$\frac{dS}{dt} = \left(\frac{Q}{V}\right)S_0 - \left(\frac{Q}{V}\right)S - \frac{\mu X}{Y} \tag{4-21}$$

Where

$S_0$ = substrate concentration in feed, g COD/L

S = substrate concentration in effluent , g COD/L

Y = yield coefficient, g VSS/g COD

Under steady-state condition, the rate of change in substrate concentration is negligible, therefore equation (4-21) can be rearranged to equation (4-22) below.

$$\frac{1}{\theta}(S_0 - S) = \mu\frac{X}{Y} \tag{4-22}$$

**c) Relationship of substrate utilization and microorganisms**

The relationship of the rate limiting substrate concentration and specific growth rate can be expressed by the Monod equation as follow.

$$\mu = \frac{\mu_m S}{(K_s + S)} \tag{4-23}$$

Substrate utilization can be expressed as

$$r_{su} = \frac{\mu_m XS}{(K_s + S)} \tag{4-24}$$

### 4.9.2 Flow Diagram

The relationship of microorganisms and substrate utilization was drawn

in the forms of flow diagram of STELLA software in Figure 4.28. It can be categorized into 3 sectors namely; (i) microorganisms sector, (ii) substrate sector and, (iii) biogas production sector. After setting up the model in flow diagram form, STELLA automatically converted basic equation. And then, the designed equations and constant values were filled up in the designed equation. After completing the model setup and fill up data, the simulation can be started immediately.

### 4.9.3 Model Equation in STELLA

The prepared flow diagram was converted to equation form. For example, equation (4-19) of microorganism was converted to

$$X(t) = X(t - dt) + (rg)dt \quad (4\text{-}25)$$

Other equations were also converted in similar manners, details of Model equation used in STELLA are shown in Appendix F.

### 4.9.4 Model Components

The model was constructed, and is displayed, in a diagrammatic form in its three sectors (microorganisms, substrate utilization and biogas production) as illustrated in Figure 4.28. This diagram shows the general structure of the model and the connections among variables, but not the specific relationships. Several variables were taken into account and could be easily modified between, or during model runs. Simulation outputs of model is displayed graphically. The full set of equations is illustrated in Appendix F.

### 4.9.5 The Example of Simple Microorganism Model

The example of STELLA flow diagram of simple microorganism model can be illustrated below with 3 phases as follow.

1) Flow Diagram: This simple model concentrated only number of

microorganism (microoganism sector) in reactor by considering the net growth rate as shown in Figure 4.28 with the assumption of 1% net growth rate.

2) The equation in STELLA format for simple microoganism model.

Microorganisms sector: X(t) = X(t - dt) + (rg) * dt

Number of microorganism (X) depended on growth rate (rg);

INIT X = 100

Initial concentration of microorganism in g/L or other unit

INFLOWS: rg = net_growth_rate_of_microorganism*X

net_growth_rate_of_microorganism = 0.01

Use constant value for net growth rate

3) Results: The example of this simple microorganism, was initiated with the 100 of microorganism, 1.00% of growth rate. The simulation was conducted for 10 years, the results of microorganism for ten years are illustrated in Table 4.10.

**Table 4.10** The results obtained from simple microorganism population model simulated by using STELLA software.

| Year | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Microorganism | 101 | 102 | 103 | 104 | 105 | 106 | 107 | 108 | 109 | 110 |

### 4.9.6 Simulation Results

1) Base run: The output from the base run (Figure 4.29) illustrates the connections and feedback of the concerned parameters. In the base run, effluent concentration was charply dropped at the first period of simulation and then close to the limit value and was slightly stable or could be said that it was steady-state which

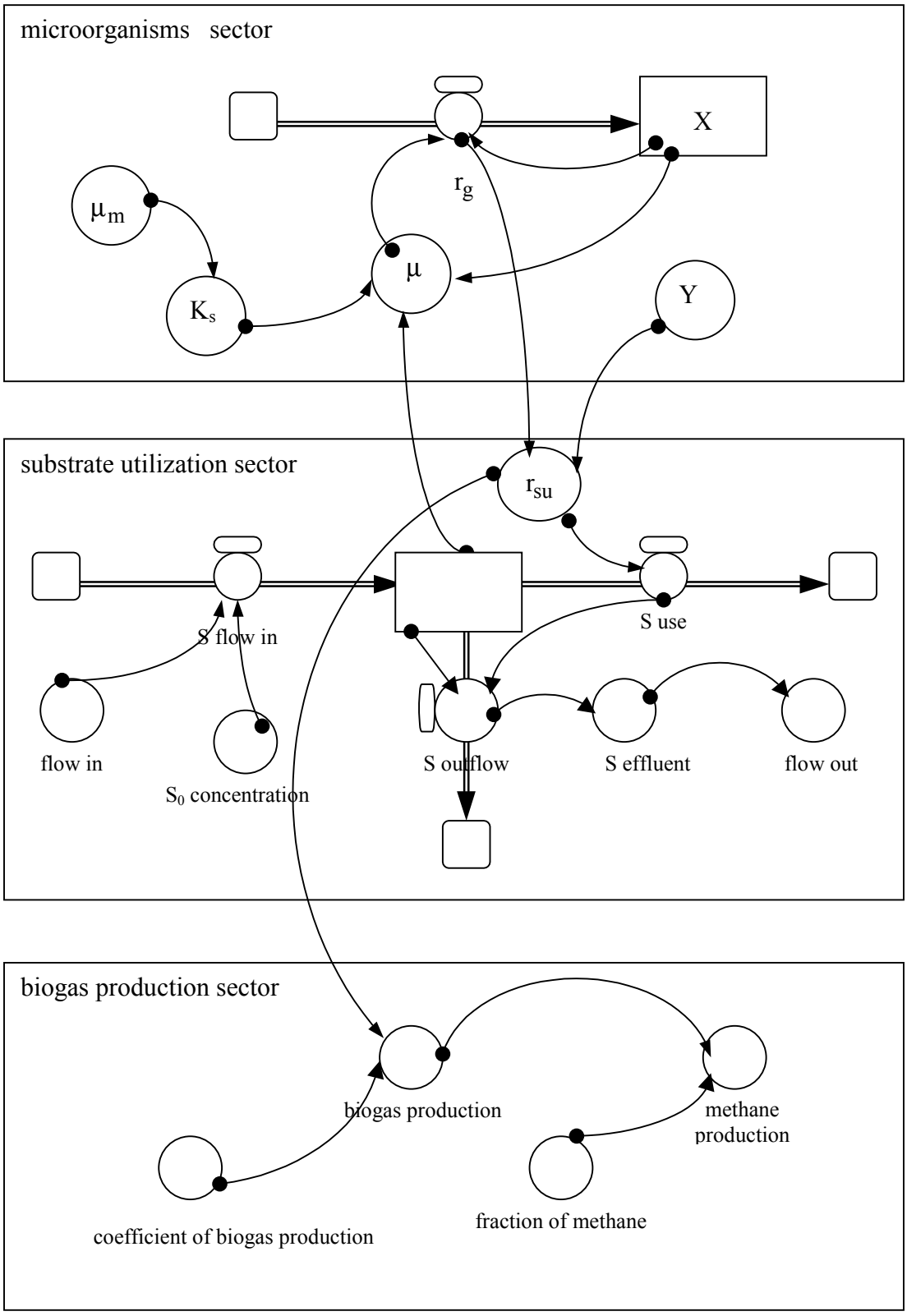



**Figure 4.28** Flow diagram of this model in STELLA format.

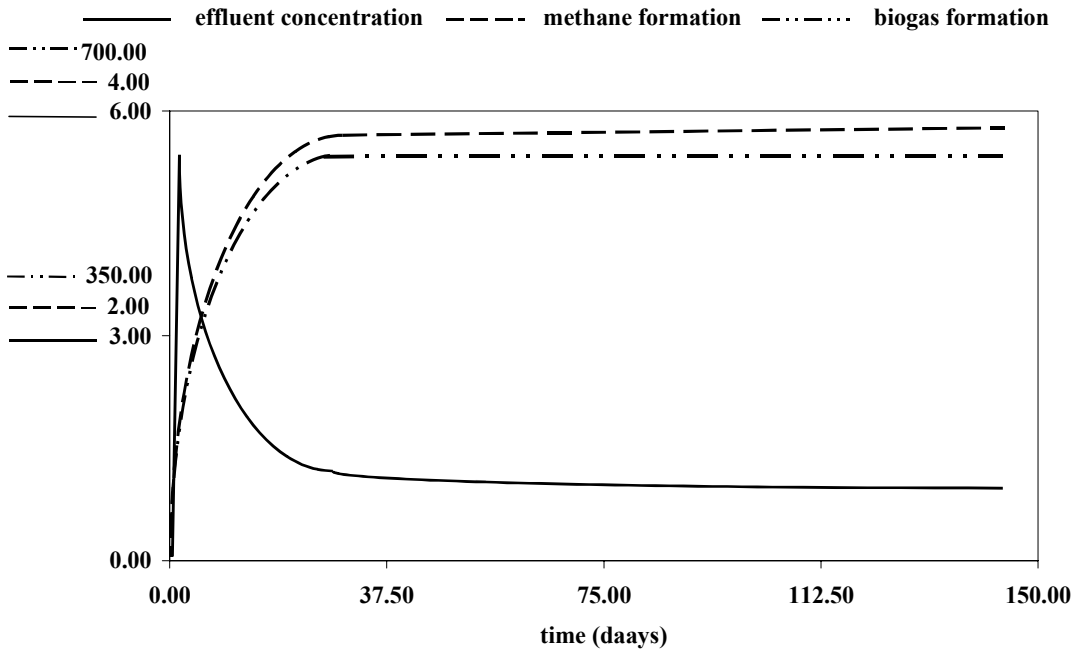



**Figure 4.29** Simulation results for base run study.

was achieved after 121 days from the start date of operation. The biogas production including methane were also simulated and were stable at steady state similar to the effluent concentration. In case of biogas production, the relationship of actual biogas production and estimated values is shown in Figure 4.30.

2) Calibration: The calibrations of constructed model were performed by least square method by using obtained data generated from run#1 (24-h HRT) and run#2 (20-h HRT) as shown in Table 4.11. It indicated that the estimated values agreed with the monitoring values with the correlation of 0.827 or 0.684 of $R^2$ and 0.813 of correlation or 0.662 $R^2$ for 24-h and 20-h HRTs, respectively. as shown in Figure 4.31 and 4.32, respectively. The ANOVA (F-test) was also performed with the significant values of 0.011 and 0.004 for run#1 and run# 2, respectively as shown in Appendix G.

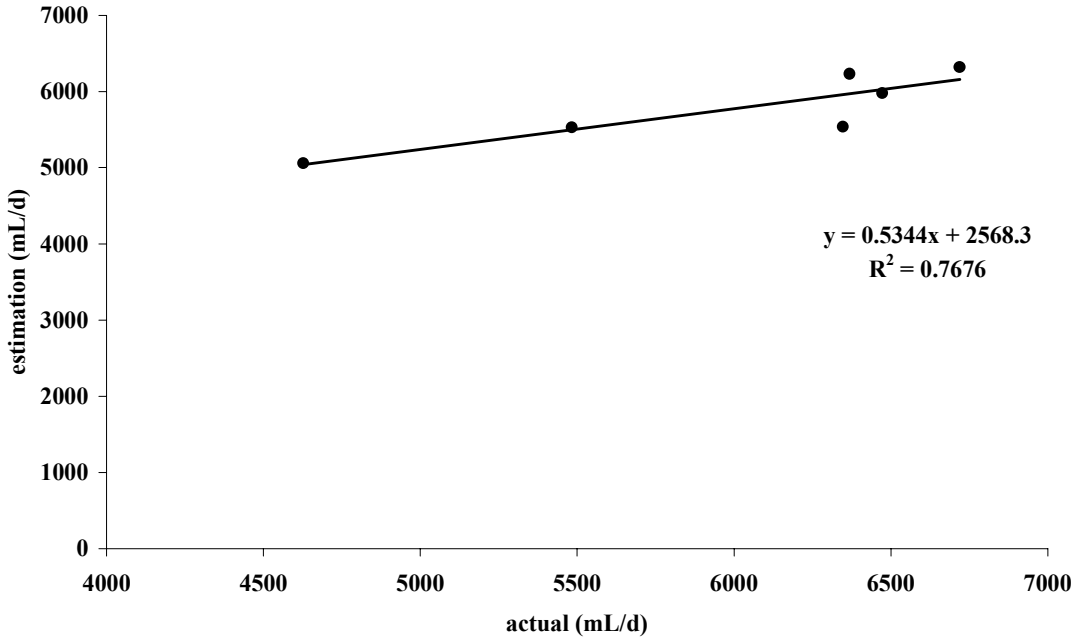



**Figure 4.30** Comparison of experimental and estimated biogas production.

**Table 4.11** The obtained data generated from run # 1 (24-h HRT) and run# 2 (20-h HRT) used for calibration of developed model.

| Run # 1 (24-h HRT) | | | Run # 2 (20-h HRT) | | |
|---|---|---|---|---|---|
| Date | Estimated COD, mg/L | Actual COD, mg/L | Date | Estimated COD, mg/L | Actual COD, mg/L |
| 18 May 02 | 123.06<br>139.47 | 126.3<br>140.3 | 7 Jul. 02 | 87.67<br>93.7 | 89.7<br>95.88 |
| 19 May 02 | 114.85<br>108.23 | 112.2<br>113.9 | 8 Jul. 02 | 70.6<br>87.0 | 82.0<br>96.29 |
| 20 May 02 | 104.58<br>139.85 | 96.3<br>126.3 | 9 Jul. 02 | 85.0 | 96.29 |
| 21 May 02 | 114.85<br>104.58 | 98.2<br>110.08 | 10 Jul. 02 | 87.375 | 89.7 |

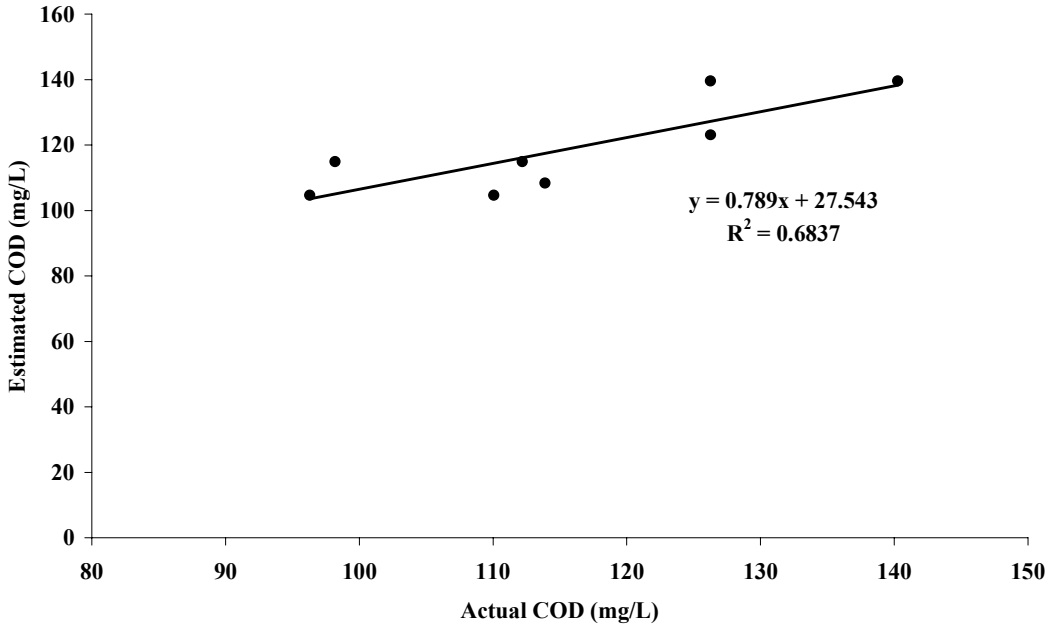



**Figure 4.31** Comparison of experimental and estimated effluent COD for model calibration of run# 1 (24-h HRT).

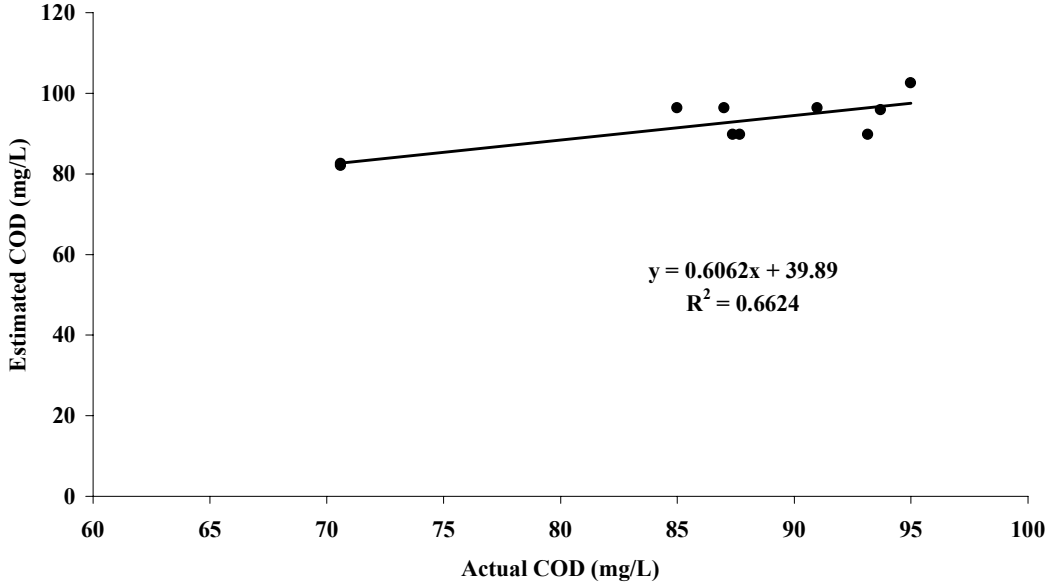



**Figure 4.32** Comparison of experimental and estimated effluent COD for model calibration of run# 2 (20-h HRT).

3) Validation: The samples taken from 16-h and 12-h HRT were used for validation (Table 4.12). The estimated COD effluent values did not agree with the monitoring values for run# 3 as shown in Figure 4.33. The significant value of 0.219 was found by using ANOVA (F-test), the details are shown in Appendix G. In case of run # 4, the estimated COD effluents agreed with the monitored concentrations as shown in Figure 4.34. The significant value was 0.028 by using ANOVA test as illustrated in Appendix G.

**Table 4.12** The obtained data generated from run # 3 (16-h HRT) and run# 4 (12-h HRT) used for validation of developed model.

| Run # 3 (16-h HRT) | | | Run # 4 (12-h HRT) | | |
|---|---|---|---|---|---|
| Date | Estimated COD, mg/L | Actual COD, mg/L | Date | Estimated COD, mg/L | Actual COD, mg/L |
| 7 Jul. 02 | 76.7 | 89.7 | 17 Oct. 02 | 263.53 | 133.3 |
| 8 Jul. 02 | 82.35 | 96.29 | 18 Oct. 02 | 244.71 | 155.5 |
| 9 Jul. 02 | 70.59 | 82.54 | 19 Oct. 02 | 357.65 | 146.1 |
| 10 Jul. 02 | 82.35 | 96.29 | 20 Oct. 02 | 361.90 | 146.1 |
| 11 Jul. 02 | 87.67 | 89.76 | 21 Oct. 02 | 371.43 | 163.0 |
| - | - | - | 22 Oct. 02 | 323.81 | 146.1 |
| - | - | - | 23 Oct. 02 | 514.29 | 179.9 |

4) Sensitivity analysis: Sensitivity analysis is a procedure, which normally performed on the completed and, at least partly validated model. It involves the exploration of the operation and performance of the model. That is in the successive runs of the model under identical conditions, the value of a parameter is

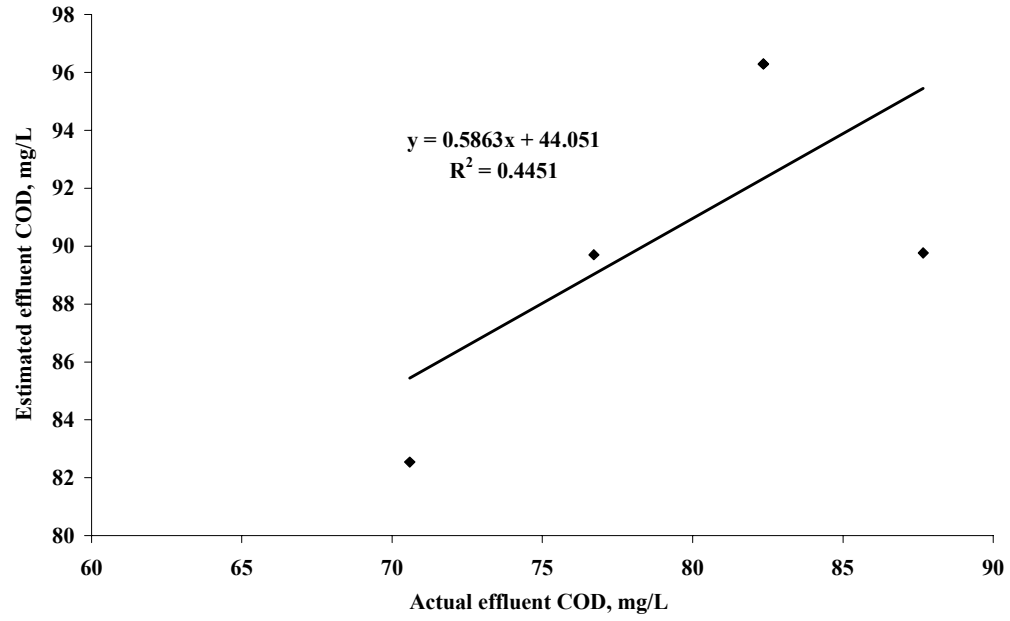



**Figure 4.33** Comparison of experimental and estimated effluent COD for model validation of run# 3 (16-h HRT).

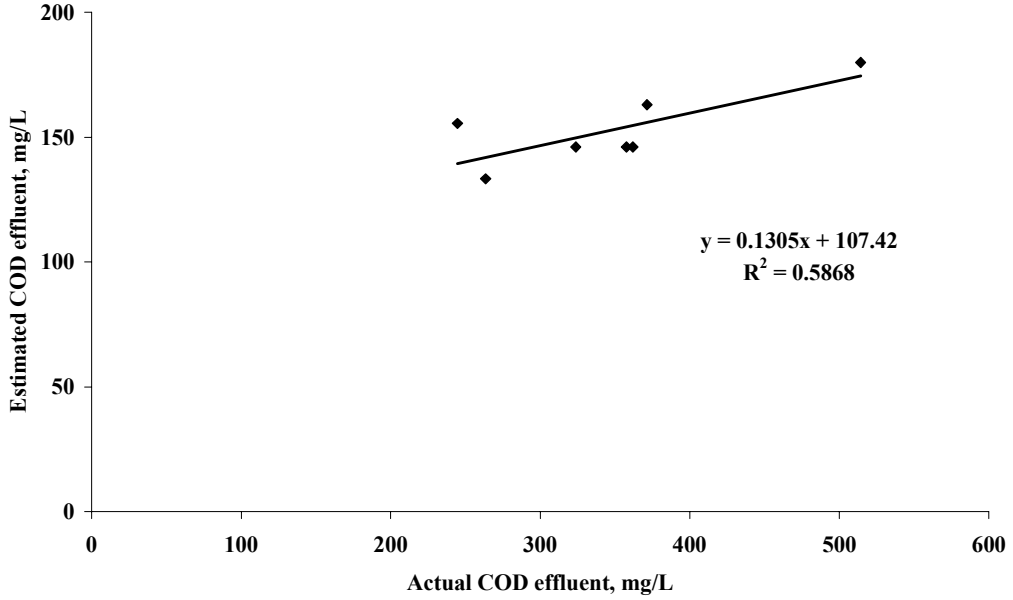



**Figure 4.34** Comparison of experimental and estimated effluent COD for model validation of run# 4 (12-h HRT).

changed. Consequently the outputs from the runs were then analyzed in order to determine whether the changed parameter values are material consequence. According to the Monod model as illustrated in equation (4-23), it can be rearranged as equation below.

$$\frac{(S_0 - S)}{t} = \frac{dS}{dt} = \frac{-kXS}{(K_s + S)} \tag{4-26}$$

The integration of above equation gave the results as follows.

$$\ln S = \ln\left[S_0 + \frac{Y(S_0 - S)S_0}{X_0}\right] + \left[\frac{(X_0 + YS_0)}{YK_s}\right]\ln\left[X_0 + \frac{Y(S_0 - S)}{X_0}\right]$$

$$-kt\frac{(X_0 + YS_0)}{K_s} \tag{4-27}$$

It can be seen that the value of S (effluent concentration) depends upon the value of $K_s$ and Y. The value of Y is estimated from $\frac{\mu_m}{k}$. In addition, an examination of the kinetic parameters of food-related wastewater had shown that Y does not vary very widely and $K_d$ did not have any appreciable effect on the effluent COD as reported by Hu et al. (2002). The sensitivity of this developed model, therefore, was performed by changing the values of $K_s$ and μm followed the factorial technique. Three factorials was employed and can be categorized into 6 cases as small, moderate and high changes as follow.

(a) Single parameter change

Small change: ±5% of $K_s$ and ±5% of $\mu_m$

Moderate change: ±10% of $K_s$ and ±10% of $\mu_m$

High change: ±15% of $K_s$ and ±15% of $\mu_m$

(b) Combination of both parameters changes

+5% of $K_s$ and +5% of $\mu_m$ and, -5% of $K_s$ and -5% of $\mu_m$

+10% of $K_s$ and +10% of $\mu_m$ and, -10% of $K_s$ and -10% of $\mu_m$

+15% of $K_s$ and +15% of $\mu_m$ and, -15% of $K_s$ and -15% of $\mu_m$

The results of above assumptions are illustrated in Table 4.13, and it showed that the changes of both parameter of $K_s$ and $\mu_m$ were not different in the effluent concentration for both increasing and decreasing of these values, however the deviations of effluent concentration were appreciable effected for single parameter change as shown in Table 4.13. The increasing of $\mu_m$ was more sensitive than the increasing of $K_s$ for +10% of increasing or moderate change, the values of deviation were 70 and 62% for +10% of $\mu_m$ and $K_s$, respectively. The patterns of single parameter changes of $K_s$ and $\mu_m$ are shown in Figure 4.35 and Figure 4.36 for ±5% and ±10%, respectively.

In case of the combination of both parameters changes, the deviations of effluent concentration were slightly change that agree with the equation (4-28) above as illustrated in Table 4.13 and Figure 4.37. It can be concluded the model will not be sensitive if the values of both parameters changes in the same direction for three levels of changes (small, moderate, and high changes) with the percentage changes less than 5%.

Moreover, the results of this study would be benefits to both design and operation of treatment process and could be used for control the plant operation. The results were agree with the equation of substrate utilization as illustrated in equation 4-32 below.

**Table 4.13** The effect on the predicted effluent COD of varying $\mu_m$ and $K_s$ by +/-5%, +/-10%, and +/-15%.

| Condition | Predicted COD, mg/L<br>$S_0$=1007 mg/L | % change |
|---|---|---|
| As modeled | 117.75 | |
| $\mu_m$ +5% | 73.13 | -34 |
| $\mu_m$ –5% | 162.37 | +34 |
| $K_s$ +5% | 160.29 | +33 |
| $K_s$ –5% | 70.72 | -36 |
| $\mu_m$ +10% | 28.52 | -70 |
| $\mu_m$ –10% | 206.99 | +70 |
| $K_s$ +10% | 198.59 | +62 |
| $K_s$ –10% | 18.95 | -76 |
| $\mu_m$ +15% | 0 | ND |
| $\mu_m$ –15% | 251.61 | 113.68 |
| $K_s$ +15% | 233.76 | 98.52 |
| $K_s$ –15% | 0 | ND |
| $\mu_m$ +5% and $K_s$ +5% | 117.80 | +0.04 |
| $\mu_m$ -5% and $K_s$ -5% | 117.69 | -0.05 |
| $\mu_m$ +10% and $K_s$ +10% | 117.47 | -0.24 |
| $\mu_m$ -10% and $K_s$ -10% | 118.10 | +0.29 |
| $\mu_m$ +15% and $K_s$ +15% | 117.37 | -0.32 |
| $\mu_m$ -15% and $K_s$ -15% | 118.27 | -0.41 |

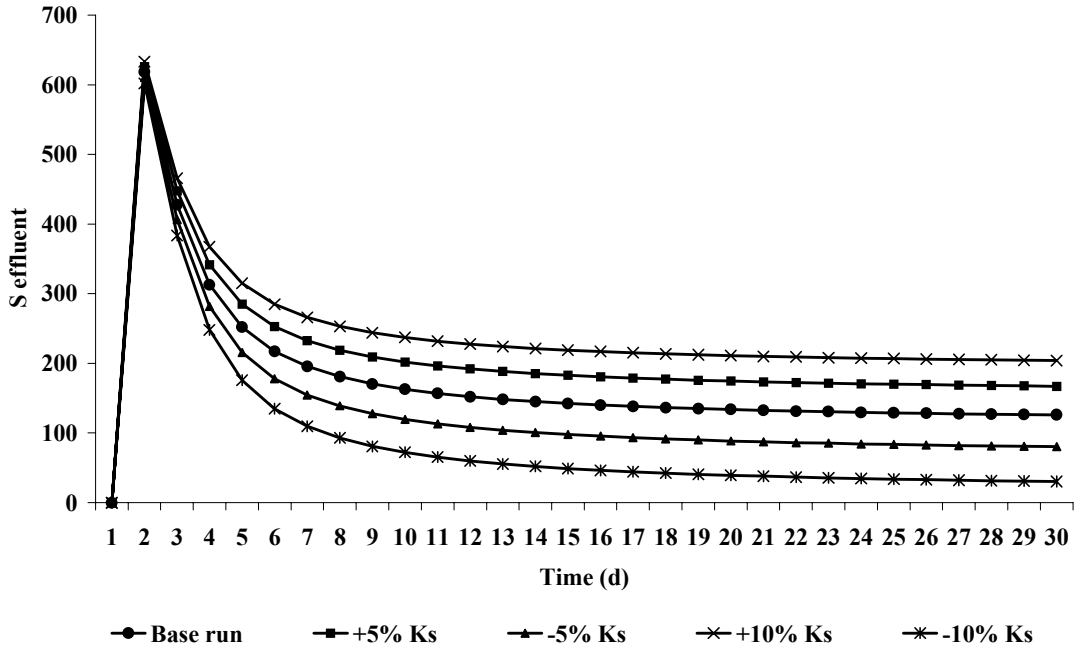



**Figure 4.35** The sensitivity analysis by changing the $K_s$ value.

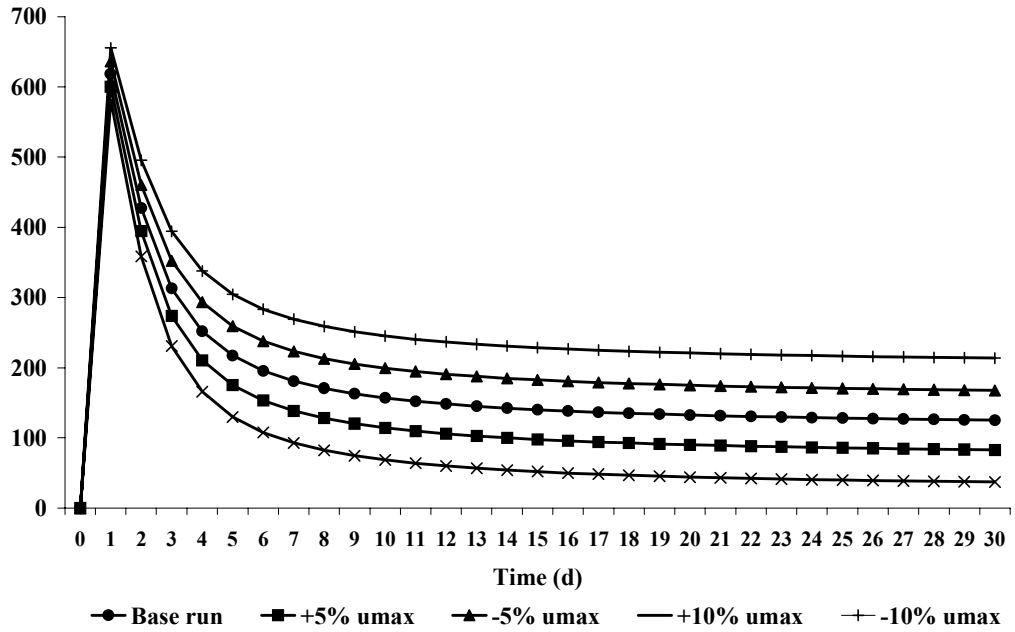



**Figure 4.36** The sensitivity analysis by changing the $\mu_m$ value.

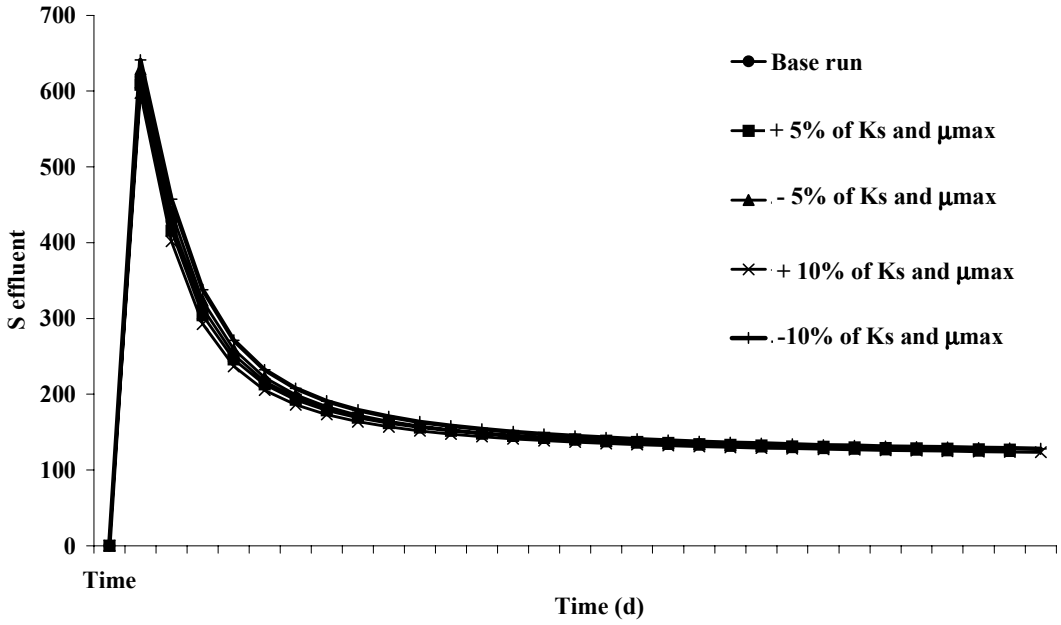



**Figure 4.37** The sensitivity analysis by changing both parameters, of $K_s$ and $\mu_m$.

$$r_{su} = \frac{\mu XS}{Y(K_S + S)} \tag{4-29}$$

The increasing of $K_s$ value, the less of substrate utilization was found that meant the effluent COD concentration would be higher. For example, the increase of 5% $K_s$, the effluent COD concentration was increased from 117.75 to 160.29 mg/L. In the similar manner, the decrease of $K_s$ would effect to the increase of substrate utilization and the consequence would be the less effluent concentrations. The decrease of 5% $K_s$ caused the decrease of effluent COD from 117.75 to 70.72 mg/L. On the other hand, the increase of $\mu_m$ as mentioned in equation (4-29) will cause the increase of substrate utilization or decrease of effluent COD. The increase of 5% $\mu_m$ influenced the decrease of COD effluent from 117.75 to 73.13 mg/L.

From Table 4.13, it can be concluded that the sensitivity levels of

increasing or decreasing of $\mu_m$ and $K_s$ greater than 5% were highly sensitive, whereas the change of +/-5% of $\mu_m$ and $K_s$ were moderately sensitive. The changes of both $\mu_m$ and $K_s$ were determined as not sensitive.

Grady et al. (1999) indicated that the pH of an anaerobic system has a strong impact on $\mu$, the results obtained from this study also indicated that the performance of reactor depended on the value of $\mu_m$. Therefore, in order to keep the good performance of reactor, the pH values should be maintained as proper values of 6.5-7.5. The study about the effect of temperature on kinetics values reported by the same researchers that the decrease of temperature from 35$^o$C to 30 and 25$^o$C caused the decrease of $\mu$ and increase of $K_s$ values. The consequence of these change caused the decrease of removal efficiency, therefore the temperature should be taken into account for the plant operation in order to achieve the good performance of reactor.

# Chapter V

# Conclusion and Recommendations

## 5.1 Conclusions

Based on this study, it appears that the use of UASB process for treating dairy wastewater is feasible, although generally the effluent may not achieve the industrial effluent standard. Other types of wastewater treatment processes should, accordingly be followed, such as aerobic treatment, wetland, land treatment process and etc. The following conclusions can be drawn from this study.

### 5.1.1 Performance of UASB Reactor for Treating Dairy Wastewater

Laboratory-scale UASB was used for treating low strength type of dairy wastewater with 66 to 92% of total COD removal efficiency for 12-h to 24-h of HRT. The suitable F/M ratios for high COD removal were found to be in the range of 0.37 to 0.6 g COD/g VSS-d and it can be used as one parameter for testing the reactor performance. The COD removal efficiency would be inferior (less than 80%) if F/M ratios exceed 0.55 kg COD/kg VSS-d. The increasing of organic loading rate or decreasing of hydraulic retention time caused the decrease of removal efficiencies of solids. Organic-nitrogen and nitrate-nitrogen were removed with the efficiency of 65-83 and 54-78%, respectively for the organic-nitrogen loading rate (NLR) from 22.9 to 54.8 g NLR/$m^3$-d, respectively. The removal of organic phosphorus were in the ranges of 68-95% at 12-h to 24-h of hydraulic retention time, while total and ortho-phosphate in influent and effluent were not different. The excellent removal efficiency

of organic-phosphorus obtained from this study ranged from 0.49 to 1.6 g PLR/$m^3$-d.

### 5.1.2 The Influence of Upflow Velocity on Granule Size

The average granule size of all upflow velocities in this study was 1,533 mm and it was not affected by the upflow velocity ranging from 0.045 to 0.090 m/hr.

### 5.1.3 Biogas Production

The average methane production in this study was 0.375 $m^3$ per kilogram of COD utilized, this value is slightly superior to the theoretical of 0.35 $m^3$ $CH_4$/kg COD. The biogas production rates were 4 to 6.8 L/d for the flow rates of 16.8 to 33.6 L/d, respectively.

### 5.1.4 Acid Distribution

The major acids found in the effluent were propionic and lactic acid. The results obtained from this study indicated that propionic acid had been little affected by hydraulic retention time, whereas lactic acid was significantly influenced by hydraulic retention time. The reaction rate of acid distribution of acetic acid to methane gas was determined as the limiting step of biogas production.

### 5.1.5 Kinetics Estimation

The kinetic coefficients obtained from this study are shown in Table 5-1. The reaction rate of acetic acid to methane gas was determined as limiting rate of dairy wastewater treatment process.

### 5.1.6 Model Formulation

The estimation of concerned parameters by using computer simulation indicated that the estimated values agreed with the results obtained from laboratory by using validation analysis. The sensitivity of this model was also performed and

**Table 5.1** The kinetic coefficients obtained from this study.

| Kinetic parameters | Unit | Value |
|---|---|---|
| k | $d^{-1}$ | 13 |
| $K_s$ | mg/L | 14.73 |
| Y | g VSS/ g COD | 0.19 |

found that it was more sensitive to $K_s$ value than $\mu_m$, and the increasing and of both parameters gave the good performance of model than the decreasing or increasing only single parameter.

## 5.2 Recommendations

Since the effluents of this process at various hydraulic retention times did not meet the industrial effluent standard, therefore if the influent concentrations are in the range similar this study, there should be some tertiary treatment processes following this system. Furthermore, the one characteristic of dairy wastewater is the high content of protein and lipid. Therefore, there should be a pretreatment process to trap oil and grease, prior to discharging wastewater to UASB process. This will be beneficial to the performance of UASB reactor in the long run. In addition, the hydrogen content in effluent should also be investigated to study in details the relationships of related parameters.

As the effluent in this study was found to contain a higher concentrations of phosphorus and nitrogen, it is recommended that some way of nutrient recovery should be explored.

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# Appendix A

## Hydraulic Study

**Table A.1** Empty-bed reactor with 4-h HRT.

| Time (min) | $\Delta t$ | $Cl^-$ (mg/L) | $\frac{C}{C_0} = C_i$ | $C_i \Delta t$ | $t_i C_i \Delta t$ | $t_i^2 C_i \Delta t$ |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0.00000 | 0.00000 | 0 | 0 |
| 10 | 10 | 0 | 0.00000 | 0.00000 | 0 | 0 |
| 20 | 10 | 0 | 0.00000 | 0.00000 | 0 | 0 |
| 30 | 10 | 0 | 0.00000 | 0.00000 | 0 | 0 |
| 40 | 10 | 4.12 | 0.00008 | 0.00082 | 0.03296 | 1.3184 |
| 50 | 10 | 12.37 | 0.00025 | 0.00247 | 0.1237 | 6.185 |
| 60 | 10 | 12.37 | 0.00025 | 0.00247 | 0.14844 | 8.9064 |
| 70 | 10 | 12.37 | 0.00025 | 0.00247 | 0.17318 | 12.1226 |
| 80 | 10 | 12.37 | 0.00025 | 0.00247 | 0.19792 | 15.8336 |
| 90 | 10 | 12.37 | 0.00025 | 0.00247 | 0.22266 | 20.0394 |
| 100 | 10 | 12.37 | 0.00025 | 0.00247 | 0.2474 | 24.74 |
| 110 | 10 | 20.62 | 0.00041 | 0.00412 | 0.45364 | 49.9004 |
| 120 | 10 | 20.62 | 0.00041 | 0.00412 | 0.49488 | 59.3856 |
| 130 | 10 | 20.62 | 0.00041 | 0.00412 | 0.53612 | 69.6956 |
| 140 | 10 | 20.62 | 0.00041 | 0.00412 | 0.57736 | 80.8304 |
| 150 | 10 | 20.62 | 0.00041 | 0.00412 | 0.6186 | 92.79 |
| 160 | 10 | 20.62 | 0.00041 | 0.00412 | 0.65984 | 105.5744 |
| 170 | 10 | 20.62 | 0.00041 | 0.00412 | 0.70108 | 119.1836 |
| 180 | 10 | 20.62 | 0.00041 | 0.00412 | 0.74232 | 133.6176 |
| 190 | 10 | 20.62 | 0.00041 | 0.00412 | 0.78356 | 148.8764 |
| 200 | 10 | 20.62 | 0.00041 | 0.00412 | 0.8248 | 164.96 |
| 210 | 10 | 20.62 | 0.00041 | 0.00412 | 0.86604 | 181.8684 |
| 220 | 10 | 20.62 | 0.00041 | 0.00412 | 0.90728 | 199.6016 |
| 230 | 10 | 20.62 | 0.00041 | 0.00412 | 0.94852 | 218.1596 |
| 240 | 10 | 20.62 | 0.00041 | 0.00412 | 0.98976 | 237.5424 |
| 250 | 10 | 53.61 | 0.00107 | 0.01072 | 2.6805 | 670.125 |
| 260 | 10 | 61.86 | 0.00124 | 0.01237 | 3.21672 | 836.3472 |
| 270 | 10 | 53.61 | 0.00107 | 0.01072 | 2.89494 | 781.6338 |
| 280 | 10 | 53.61 | 0.00107 | 0.01072 | 3.00216 | 840.6048 |
| 290 | 10 | 53.61 | 0.00107 | 0.01072 | 3.10938 | 901.7202 |
| 300 | 10 | 53.61 | 0.00107 | 0.01072 | 3.2166 | 964.98 |
| 310 | 10 | 53.61 | 0.00107 | 0.01072 | 3.32382 | 1030.3842 |
| 320 | 10 | 53.61 | 0.00107 | 0.01072 | 3.43104 | 1097.9328 |
| 330 | 10 | 53.61 | 0.00107 | 0.01072 | 3.53826 | 1167.6258 |
| 340 | 10 | 53.61 | 0.00107 | 0.01072 | 3.64548 | 1239.4632 |
| 350 | 10 | 53.61 | 0.00107 | 0.01072 | 3.7527 | 1313.445 |
| 360 | 10 | 20.62 | 0.00041 | 0.00412 | 1.48464 | 534.4704 |
| 370 | 10 | 20.62 | 0.00041 | 0.00412 | 1.52588 | 564.5756 |
| 380 | 10 | 20.62 | 0.00041 | 0.00412 | 1.56712 | 595.5056 |
| 390 | 10 | 20.62 | 0.00041 | 0.00412 | 1.60836 | 627.2604 |
| 400 | 10 | 20.62 | 0.00041 | 0.00412 | 1.6496 | 659.84 |
| 410 | 10 | 20.62 | 0.00041 | 0.00412 | 1.69084 | 693.2444 |
| 420 | 10 | 20.62 | 0.00041 | 0.00412 | 1.73208 | 727.4736 |
| 430 | 10 | 20.62 | 0.00041 | 0.00412 | 1.77332 | 762.5276 |
| 440 | 10 | 20.62 | 0.00041 | 0.00412 | 1.81456 | 798.4064 |

**Table A.1** Empty-bed reactor with 4-h HRT. (continued)

| Time (min) | $\Delta t$ | $Cl^-$ (mg/L) | $\frac{C}{C_0} = C_i$ | $C_i \Delta t$ | $t_i C_i \Delta t$ | $t_i^2 C_i \Delta t$ |
|---|---|---|---|---|---|---|
| 450 | 10 | 20.62 | 0.00041 | 0.00412 | 1.8558 | 835.11 |
| 460 | 10 | 20.62 | 0.00041 | 0.00412 | 1.89704 | 872.6384 |
| 470 | 10 | 20.62 | 0.00041 | 0.00412 | 1.93828 | 910.9916 |
| 480 | 10 | 20.62 | 0.00041 | 0.00412 | 1.97952 | 950.1696 |
| Sum | 480 | 1233.04 | 0.02466 | 0.24661 | 69.5787 | 22327.607 |

$$\text{theoritical retention time } (\theta) = \frac{\text{effective volume of reactor}}{\text{flow rate}}$$

$$= \frac{16.8 \text{ L}}{70 \ \frac{\text{mL}}{\text{min}}} = 240 \text{ minutes}$$

$$T_{mean} = \frac{\sum t_i c_i \Delta t_i}{\sum c_i \Delta t_i} = \frac{65.5787}{0.24661}$$

$$= 282.14 \text{ minutes}$$

$$\sigma^2 = \frac{\sum t_i^2 c_i \Delta t_i}{\sum c_i \Delta t_i} - T_{mean}^2$$

$$= \frac{22327.607}{0.2466} - 282.14^2$$

$$= 10934.2347$$

$$2d + 8d^2 = \frac{\sigma^2}{T_{mean}^2} = \frac{10934.2347}{282.14^2}$$

$$= 0.13735678$$

$$d = 0.0561$$

$$d = \frac{D}{\mu L} = \text{Dispersion number}$$

**Table A.2** Empty-bed reactor with 12-h HRT.

| Time (min) | $\Delta t$ | $Cl^-$ (mg/L) | $\frac{C}{C_0} = C_i$ | $C_i \Delta t$ | $t_i C_i \Delta t$ | $t_i^2 C_i \Delta t$ |
|---|---|---|---|---|---|---|
| 0 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 10 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 20 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 30 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 40 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 50 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 60 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 70 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 80 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 90 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 100 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 110 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 120 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 130 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 140 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 150 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 160 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 170 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 180 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 190 | 10 | 24.99 | 0.0005 | 0.00 | 0.95 | 180.44 |
| 200 | 10 | 0.00 | 0.0000 | 0.00 | 0.00 | 0.00 |
| 210 | 10 | 12.50 | 0.0002 | 0.00 | 0.52 | 110.22 |
| 220 | 10 | 24.99 | 0.0005 | 0.00 | 1.10 | 241.92 |
| 230 | 10 | 24.99 | 0.0005 | 0.00 | 1.15 | 264.42 |
| 240 | 10 | 24.99 | 0.0005 | 0.00 | 1.20 | 287.91 |
| 250 | 10 | 24.99 | 0.0005 | 0.00 | 1.25 | 312.40 |
| 260 | 10 | 24.99 | 0.0005 | 0.00 | 1.30 | 337.90 |
| 270 | 10 | 24.99 | 0.0005 | 0.00 | 1.35 | 364.39 |
| 280 | 10 | 24.99 | 0.0005 | 0.00 | 1.40 | 391.88 |
| 290 | 10 | 24.99 | 0.0005 | 0.00 | 1.45 | 420.37 |
| 300 | 10 | 37.49 | 0.0007 | 0.01 | 2.25 | 674.79 |
| 310 | 10 | 37.49 | 0.0007 | 0.01 | 2.32 | 720.53 |
| 320 | 10 | 37.49 | 0.0007 | 0.01 | 2.40 | 767.76 |
| 330 | 10 | 37.49 | 0.0007 | 0.01 | 2.47 | 816.50 |
| 340 | 10 | 37.49 | 0.0007 | 0.01 | 2.55 | 866.73 |
| 350 | 10 | 37.49 | 0.0007 | 0.01 | 2.62 | 918.47 |
| 360 | 10 | 49.98 | 0.0010 | 0.01 | 3.60 | 1295.60 |
| 370 | 10 | 49.98 | 0.0010 | 0.01 | 3.70 | 1368.58 |
| 380 | 10 | 49.98 | 0.0010 | 0.01 | 3.80 | 1443.55 |
| 390 | 10 | 74.98 | 0.0015 | 0.01 | 5.85 | 2280.79 |
| 400 | 10 | 74.98 | 0.0015 | 0.01 | 6.00 | 2399.26 |
| 410 | 10 | 84.97 | 0.0017 | 0.02 | 6.97 | 2856.81 |
| 420 | 10 | 89.97 | 0.0018 | 0.02 | 7.56 | 3174.22 |
| 430 | 10 | 94.97 | 0.0019 | 0.02 | 8.17 | 3512.01 |
| 440 | 10 | 99.97 | 0.0020 | 0.02 | 8.80 | 3870.80 |

**Table A.2** Empty-bed reactor with 12-h HRT. (continued)

| Time (min) | $\Delta t$ | $Cl^-$ (mg/L) | $\frac{C}{C_0} = C_i$ | $C_i \Delta t$ | $t_i C_i \Delta t$ | $t_i^2 C_i \Delta t$ |
|---|---|---|---|---|---|---|
| 450 | 10 | 100.97 | 0.0020 | 0.02 | 9.09 | 4089.23 |
| 460 | 10 | 98.97 | 0.0020 | 0.02 | 9.11 | 4188.38 |
| 470 | 10 | 105.47 | 0.0021 | 0.02 | 9.91 | 4659.55 |
| 480 | 10 | 104.97 | 0.0021 | 0.02 | 10.08 | 4836.90 |
| 490 | 10 | 110.97 | 0.0022 | 0.02 | 10.87 | 5328.57 |
| 500 | 10 | 111.97 | 0.0022 | 0.02 | 11.20 | 5598.26 |
| 510 | 10 | 113.96 | 0.0023 | 0.02 | 11.62 | 5928.44 |
| 520 | 10 | 112.47 | 0.0022 | 0.02 | 11.70 | 6082.11 |
| 530 | 10 | 112.47 | 0.0022 | 0.02 | 11.92 | 6318.29 |
| 540 | 10 | 112.96 | 0.0023 | 0.02 | 12.20 | 6588.12 |
| 550 | 10 | 112.96 | 0.0023 | 0.02 | 12.43 | 6834.38 |
| 560 | 10 | 112.96 | 0.0023 | 0.02 | 12.65 | 7085.16 |
| 570 | 10 | 114.96 | 0.0023 | 0.02 | 13.11 | 7470.38 |
| 580 | 10 | 115.96 | 0.0023 | 0.02 | 13.45 | 7802.06 |
| 590 | 10 | 117.46 | 0.0023 | 0.02 | 13.86 | 8177.81 |
| 600 | 10 | 117.96 | 0.0024 | 0.02 | 14.16 | 8493.37 |
| 610 | 10 | 118.96 | 0.0024 | 0.02 | 14.51 | 8853.23 |
| 620 | 10 | 119.46 | 0.0024 | 0.02 | 14.81 | 9184.31 |
| 630 | 10 | 119.46 | 0.0024 | 0.02 | 15.05 | 9482.97 |
| 640 | 10 | 119.96 | 0.0024 | 0.02 | 15.36 | 9827.35 |
| 650 | 10 | 124.96 | 0.0025 | 0.02 | 16.24 | 10559.23 |
| 660 | 10 | 129.46 | 0.0026 | 0.03 | 17.09 | 11278.54 |
| 670 | 10 | 144.46 | 0.0029 | 0.03 | 19.36 | 12969.19 |
| 680 | 10 | 144.96 | 0.0029 | 0.03 | 19.71 | 13405.44 |
| 690 | 10 | 145.95 | 0.0029 | 0.03 | 20.14 | 13897.81 |
| 700 | 10 | 147.45 | 0.0029 | 0.03 | 20.64 | 14450.52 |
| 710 | 10 | 149.90 | 0.0030 | 0.03 | 21.29 | 15113.27 |
| 720 | 10 | 149.95 | 0.0030 | 0.03 | 21.59 | 15547.18 |
| 730 | 10 | 1973.79 | 0.0395 | 0.39 | 288.17 | 210366.32 |
| 740 | 10 | 2723.31 | 0.0545 | 0.54 | 403.05 | 298256.42 |
| 750 | 10 | 2973.18 | 0.0595 | 0.59 | 445.98 | 334482.53 |
| 760 | 10 | 2973.18 | 0.0595 | 0.59 | 451.92 | 343461.53 |
| 770 | 10 | 2923.19 | 0.0585 | 0.58 | 450.17 | 346632.29 |
| 780 | 10 | 2898.20 | 0.0580 | 0.58 | 452.12 | 352653.13 |
| 790 | 10 | 2898.20 | 0.0580 | 0.58 | 457.92 | 361753.48 |
| 800 | 10 | 2898.20 | 0.0580 | 0.58 | 463.71 | 370969.76 |
| 810 | 10 | 2898.20 | 0.0580 | 0.58 | 469.51 | 380301.97 |
| 820 | 10 | 2898.20 | 0.0580 | 0.58 | 475.31 | 389750.11 |
| 830 | 10 | 2773.29 | 0.0555 | 0.55 | 460.37 | 382103.90 |
| 840 | 10 | 2698.31 | 0.0540 | 0.54 | 453.32 | 380785.97 |
| 850 | 10 | 2648.38 | 0.0530 | 0.53 | 450.22 | 382690.73 |
| 860 | 10 | 2573.40 | 0.0515 | 0.51 | 442.63 | 380657.62 |
| 870 | 10 | 2523.42 | 0.0505 | 0.50 | 439.07 | 381994.94 |
| 880 | 10 | 2448.49 | 0.0490 | 0.49 | 430.93 | 379222.24 |
| 890 | 10 | 2423.50 | 0.0485 | 0.48 | 431.38 | 383930.63 |

**Table A.2** Empty-bed reactor with 12-h HRT. (continued)

| Time (min) | $\Delta t$ | $Cl^-$ (mg/L) | $\frac{C}{C_0} = C_i$ | $C_i \Delta t$ | $t_i C_i \Delta t$ | $t_i^2 C_i \Delta t$ |
|---|---|---|---|---|---|---|
| 900 | 10 | 2348.52 | 0.0470 | 0.47 | 422.73 | 380460.52 |
| 910 | 10 | 2273.59 | 0.0455 | 0.45 | 413.79 | 376552.80 |
| 920 | 10 | 2248.60 | 0.0450 | 0.45 | 413.74 | 380643.47 |
| 930 | 10 | 2198.62 | 0.0440 | 0.44 | 408.94 | 380316.98 |
| 940 | 10 | 2173.68 | 0.0435 | 0.43 | 408.65 | 384132.01 |
| 950 | 10 | 2148.68 | 0.0430 | 0.43 | 408.25 | 387837.41 |
| 960 | 10 | 2123.69 | 0.0425 | 0.42 | 407.75 | 391438.81 |
| 970 | 10 | 2098.70 | 0.0420 | 0.42 | 407.15 | 394933.22 |
| 980 | 10 | 2073.71 | 0.0415 | 0.41 | 406.45 | 398317.63 |
| 990 | 10 | 2023.72 | 0.0405 | 0.40 | 400.70 | 396690.07 |
| 1000 | 10 | 1998.78 | 0.0400 | 0.40 | 399.76 | 399756.04 |
| 1010 | 10 | 1998.78 | 0.0400 | 0.40 | 403.75 | 407791.13 |
| 1020 | 10 | 1998.78 | 0.0400 | 0.40 | 407.75 | 415906.18 |
| 1030 | 10 | 1923.80 | 0.0385 | 0.38 | 396.30 | 408192.61 |
| 1040 | 10 | 1798.89 | 0.0360 | 0.36 | 374.17 | 389136.35 |
| 1050 | 10 | 1748.91 | 0.0350 | 0.35 | 367.27 | 385634.14 |
| 1060 | 10 | 1748.91 | 0.0350 | 0.35 | 370.77 | 393014.53 |
| 1070 | 10 | 1748.91 | 0.0350 | 0.35 | 374.27 | 400464.88 |
| 1080 | 10 | 1698.92 | 0.0340 | 0.34 | 366.97 | 396324.80 |
| 1090 | 10 | 1673.98 | 0.0335 | 0.33 | 364.93 | 397771.34 |
| 1100 | 10 | 1648.99 | 0.0330 | 0.33 | 362.78 | 399055.25 |
| 1110 | 10 | 1648.99 | 0.0330 | 0.33 | 366.08 | 406343.78 |
| 1120 | 10 | 1599.00 | 0.0320 | 0.32 | 358.18 | 401158.16 |
| 1130 | 10 | 1599.00 | 0.0320 | 0.32 | 361.37 | 408353.68 |
| 1140 | 10 | 1549.02 | 0.0310 | 0.31 | 353.18 | 402621.19 |
| 1150 | 10 | 1524.08 | 0.0305 | 0.30 | 350.54 | 403118.47 |
| 1160 | 10 | 1524.08 | 0.0305 | 0.30 | 353.59 | 410159.71 |
| 1170 | 10 | 1499.09 | 0.0300 | 0.30 | 350.79 | 410419.53 |
| 1180 | 10 | 1449.10 | 0.0290 | 0.29 | 341.99 | 403545.55 |
| 1190 | 10 | 1449.10 | 0.0290 | 0.29 | 344.89 | 410414.28 |
| 1200 | 10 | 1399.12 | 0.0280 | 0.28 | 335.79 | 402945.45 |
| 1210 | 10 | 1374.12 | 0.0275 | 0.27 | 332.54 | 402370.96 |
| 1220 | 10 | 1299.20 | 0.0260 | 0.26 | 317.00 | 386745.00 |
| 1230 | 10 | 1299.20 | 0.0260 | 0.26 | 319.60 | 393111.07 |
| 1240 | 10 | 1249.21 | 0.0250 | 0.25 | 309.80 | 384157.87 |
| 1250 | 10 | 1199.28 | 0.0240 | 0.24 | 299.82 | 374774.41 |
| 1260 | 10 | 1149.29 | 0.0230 | 0.23 | 289.62 | 364923.71 |
| 1270 | 10 | 1124.30 | 0.0225 | 0.22 | 285.57 | 362677.13 |
| 1280 | 10 | 1024.38 | 0.0205 | 0.20 | 262.24 | 335669.61 |
| 1290 | 10 | 1049.32 | 0.0210 | 0.21 | 270.73 | 349236.22 |
| 1300 | 10 | 1024.38 | 0.0205 | 0.20 | 266.34 | 346241.23 |
| 1310 | 10 | 1036.88 | 0.0207 | 0.21 | 271.66 | 355877.43 |
| 1320 | 10 | 1024.38 | 0.0205 | 0.20 | 270.44 | 356976.76 |
| 1330 | 10 | 999.39 | 0.0200 | 0.20 | 265.84 | 353564.23 |

**Table A.2** Empty-bed reactor with 12-h HRT. (continued)

| Time (min) | $\Delta t$ | $Cl^-$ (mg/L) | $\frac{C}{C_0} = C_i$ | $C_i \Delta t$ | $t_i C_i \Delta t$ | $t_i^2 C_i \Delta t$ |
|---|---|---|---|---|---|---|
| 1340 | 10 | 974.40 | 0.0195 | 0.19 | 261.14 | 349925.75 |
| 1350 | 10 | 924.41 | 0.0185 | 0.18 | 249.59 | 336948.66 |
| 1360 | 10 | 974.40 | 0.0195 | 0.19 | 265.04 | 360449.25 |
| 1370 | 10 | 949.41 | 0.0190 | 0.19 | 260.14 | 356387.87 |
| 1380 | 10 | 949.41 | 0.0190 | 0.19 | 262.04 | 361609.60 |
| 1390 | 10 | 941.21 | 0.0188 | 0.19 | 261.66 | 363701.65 |
| 1400 | 10 | 940.21 | 0.0188 | 0.19 | 263.26 | 368561.71 |
| 1410 | 10 | 929.71 | 0.0186 | 0.19 | 262.18 | 369671.97 |
| 1420 | 10 | 930.71 | 0.0186 | 0.19 | 264.32 | 375337.29 |
| 1430 | 10 | 929.21 | 0.0186 | 0.19 | 265.75 | 380029.06 |
| 1440 | 10 | 926.71 | 0.0185 | 0.19 | 266.89 | 384326.26 |
| 1450 | 10 | 926.21 | 0.0185 | 0.19 | 268.60 | 389472.48 |
| 1460 | 10 | 925.71 | 0.0185 | 0.19 | 270.31 | 394649.94 |
| 1470 | 10 | 924.71 | 0.0185 | 0.18 | 271.87 | 399642.57 |
| 1480 | 10 | 922.21 | 0.0184 | 0.18 | 272.98 | 404003.52 |
| 1490 | 10 | 920.71 | 0.0184 | 0.18 | 274.37 | 408815.65 |
| 1500 | 10 | 920.21 | 0.0184 | 0.18 | 276.06 | 414096.59 |
| 1510 | 10 | 918.22 | 0.0184 | 0.18 | 277.30 | 418724.53 |
| 1520 | 10 | 917.22 | 0.0183 | 0.18 | 278.83 | 423826.97 |
| 1530 | 10 | 914.72 | 0.0183 | 0.18 | 279.90 | 428251.90 |
| Sum | 1540 | 141123.8 | 2.822476 | 28.22 | 28866.33 | 31112150.69 |

$$\text{theoritical retention time } (\theta) = \frac{\text{effective volume of reactor}}{\text{flow rate}}$$

$$= \frac{16.8 \text{ L}}{33.4 \frac{\text{mL}}{\text{min}}} = 720 \text{ minutes}$$

$$T_{mean} = \frac{\sum t_i c_i \Delta t_i}{\sum c_i \Delta t_i} = \frac{22866.33}{28.22}$$

$$= 1022.73 \text{ minutes}$$

$$\sigma^2 = \frac{\sum t_i^2 c_i \Delta t_i}{\sum c_i \Delta t_i} - T_{mean}^2$$

$$= \frac{3111215.69}{28.22} - 1022.73^2 = 56321.33$$

$$2d + 8d^2 = \frac{\sigma^2}{T_{mean}^2} = \frac{56321.33}{1022.73^2}$$

$$= 0.053846$$

$$d = 0.0244$$

**Table A.3** The tracer study for 4-h HRT sludge-containing reactor.

| Time (min) | $\Delta t$ | $Cl^-$ (mg/L) | $\frac{C}{C_0} = C_i$ | $C_i \Delta t$ | $t_i C_i \Delta t$ | $t_i^2 C_i \Delta t$ |
|---|---|---|---|---|---|---|
| 0 | 0 | | 0.00000 | 0.00000 | 0 | 0 |
| 5 | 5 | | 0.00003 | 0.00013 | 0.00066 | 0.0033 |
| 10 | 5 | | 0.00003 | 0.00013 | 0.00132 | 0.0132 |
| 15 | 5 | | 0.00003 | 0.00013 | 0.00198 | 0.0297 |
| 20 | 5 | | 0.08000 | 0.40001 | 8.00016 | 160.0032 |
| 25 | 5 | | 0.05001 | 0.25005 | 6.2513625 | 156.284063 |
| 30 | 5 | | 0.04001 | 0.20007 | 6.0021 | 180.063 |
| 35 | 5 | | 0.05001 | 0.25005 | 8.7519075 | 306.316763 |
| 40 | 5 | | 0.12999 | 0.64993 | 25.99722 | 1039.8888 |
| 45 | 5 | | 0.00003 | 0.00013 | 0.00594 | 0.2673 |
| 50 | 5 | | 0.05001 | 0.25005 | 12.502725 | 625.13625 |
| 55 | 5 | | 0.05001 | 0.25005 | 13.7529975 | 756.414863 |
| 60 | 5 | | 0.06001 | 0.30004 | 18.00234 | 1080.1404 |
| 65 | 5 | | 0.06001 | 0.30004 | 19.502535 | 1267.66478 |
| 70 | 5 | | 0.07000 | 0.35002 | 24.501645 | 1715.11515 |
| 75 | 5 | | 0.12999 | 0.64993 | 48.7447875 | 3655.85906 |
| 80 | 5 | | 0.11999 | 0.59995 | 47.99568 | 3839.6544 |
| 85 | 5 | | 0.12999 | 0.64993 | 55.2440925 | 4695.74786 |
| 90 | 5 | | 0.16997 | 0.84987 | 76.488165 | 6883.93485 |
| 95 | 5 | | 0.12999 | 0.64993 | 61.7433975 | 5865.62276 |
| 100 | 5 | | 0.17997 | 0.89985 | 89.9853 | 8998.53 |
| 105 | 5 | | 0.17997 | 0.89985 | 94.484565 | 9920.87933 |
| 110 | 5 | | 0.18997 | 0.94984 | 104.482125 | 11493.0338 |
| 115 | 5 | | 0.20996 | 1.04981 | 120.727748 | 13883.691 |
| 120 | 5 | 10.00 | 0.19996 | 0.99982 | 119.97864 | 14397.4368 |
| 125 | 5 | 12.00 | 0.23995 | 1.19976 | 149.97 | 18746.25 |
| 130 | 5 | 11.00 | 0.21996 | 1.09979 | 142.97283 | 18586.4679 |
| 175 | 5 | 6.00 | 0.11999 | 0.59995 | 104.99055 | 18373.3463 |
| 180 | 5 | 6.00 | 0.11999 | 0.59995 | 107.99028 | 19438.2504 |
| 185 | 5 | 6.50 | 0.12999 | 0.64993 | 120.237143 | 22243.8714 |
| 190 | 5 | 6.00 | 0.11999 | 0.59995 | 113.98974 | 21658.0506 |
| 195 | 5 | 5.50 | 0.10999 | 0.54996 | 107.242493 | 20912.286 |
| 200 | 5 | 4.50 | 0.09000 | 0.44999 | 89.9985 | 17999.7 |
| 205 | 5 | 4.00 | 0.08000 | 0.40001 | 82.00164 | 16810.3362 |
| 210 | 5 | 5.00 | 0.10000 | 0.49998 | 104.99517 | 22048.9857 |

**Table A.3** The tracer study for 4-h HRT sludge-containing reactor. (continued)

| Time (min) | $\Delta t$ | $Cl^-$ (mg/L) | $\frac{C}{C_0} = C_i$ | $C_i \Delta t$ | $t_i C_i \Delta t$ | $t_i^2 C_i \Delta t$ |
|---|---|---|---|---|---|---|
| 215 | 5 | 3.00 | 0.06001 | 0.30004 | 64.508385 | 13869.3028 |
| 220 | 5 | 3.50 | 0.07000 | 0.35002 | 77.00517 | 16941.1374 |
| 225 | 5 | 3.00 | 0.06001 | 0.30004 | 67.508775 | 15189.4744 |
| 230 | 5 | 1.00 | 0.02002 | 0.10010 | 23.02323 | 5295.3429 |
| 235 | 5 | 1.00 | 0.02002 | 0.10010 | 23.523735 | 5528.07773 |
| 240 | 5 | 1.00 | 0.02002 | 0.10010 | 24.02424 | 5765.8176 |
| 245 | 5 | 1.00 | 0.02002 | 0.10010 | 24.524745 | 6008.56253 |
| 250 | 5 | 1.00 | 0.02002 | 0.10010 | 25.02525 | 6256.3125 |
| 255 | 5 | 0.50 | 0.01002 | 0.05012 | 12.7797075 | 3258.82541 |
| 260 | 5 | 0.50 | 0.01002 | 0.05012 | 13.03029 | 3387.8754 |
| 270 | 5 | 0.00 | 0.00003 | 0.00013 | 0.03564 | 9.6228 |
| 275 | 5 | 0.00 | 0.00003 | 0.00013 | 0.0363 | 9.9825 |
| Sum | 265 | 269.4864 | 5.3897 | 26.9486 | 3553.4128 | 538091.216 |

$$\text{theoritical retention time } (\theta) = \frac{\text{effective volume of reactor}}{\text{flow rate}}$$

$$= \frac{16.8 \text{ L}}{70 \ \frac{\text{mL}}{\text{min}}} = 240 \text{ minutes}$$

$$T_{mean} = \frac{\sum t_i c_i \Delta t_i}{\sum c_i \Delta t_i} = \frac{3553.41}{26.95}$$

$$= 131.86 \text{ minutes}$$

$$\sigma^2 = \frac{\sum t_i^2 c_i \Delta t_i}{\sum c_i \Delta t_i} - T_{mean}^2$$

$$= \frac{538091.22}{26.95} - 131.86^2 = 2580.56863$$

$$2d + 8d^2 = \frac{\sigma^2}{T_{mean}^2} = \frac{280.57}{131.86^2}$$

$$= 0.14819$$

$$d = 0.059$$

# Appendix B

## Laboratory Results

**Table B.1** COD removal efficiency VS hydraulic retention times (HRT) and organic loading rate (OLR).

| HRT (h) | OLR (kgCOD/ $m^3$.d) | Influent COD (mg/L) | | Effluent COD (mg/L) | | % COD Removal | |
|---|---|---|---|---|---|---|---|
| | | Total | Filtrate | Total | Filtrate | Total | Filtrate |
| 12 | 2.07 | 1033.7 | 518.30 | 351.10 | 226.60 | 66.00 | 56.30 |
| 16 | 1.28 | 853.2 | 657.00 | 84.16 | 34.78 | 90.10 | 94.70 |
| 20 | 1.02 | 853.2 | 657.00 | 65.70 | 29.00 | 92.30 | 95.60 |
| 24 | 1.01 | 1006.9 | 845.79 | 106.74 | 45.37 | 89.40 | 94.60 |

**Table B.2** The F/M ratio versus the percentage of COD removed at various retention times.

| HRT, h | F/M ratio | % removal of COD |
|---|---|---|
| 12 | 0.73 | 66.0 |
| 16 | 0.46 | 90.1 |
| 20 | 0.37 | 92.3 |
| 24 | 0.56 | 89.4 |

**Table B.3** BOD removal efficiencies at various hydraulic retention times.

| HRT | Influent BOD | Effluent BOD | % removal |
|---|---|---|---|
| 12 | 585.00 | 46.5 | 92.05 |
| 16 | 580.00 | 19.50 | 96.60 |
| 20 | 580.00 | 17.40 | 97.00 |
| 24 | 851.25 | 84.35 | 90.10 |

**Table B.4** The influent and effluent of nitrogen constituents at each HRTs and nitrogen loading rate (NLR).

| HRT (h) | Influent, mg/L | | | | Effluent, mg/L | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | TN | TKN | $NO_3^-$-N | Org.-N | TN | TKN | $NH_3$ | $NO_3^-$-N | Org.-N |
| 12 | 27.41 | 27.3 | 0.1 | 27.3 | 19.79 | 19.7 | 10.1 | 0.046 | 9.64 |
| 16 | 19.25 | 18.4 | 0.88 | 18.4 | 19.51 | 19.18 | 16.07 | 0.33 | 3.11 |
| 20 | 19.25 | 18.4 | 0.88 | 18.4 | 19.28 | 19.09 | 15.93 | 0.19 | 3.16 |
| 24 | 22.97 | 22.2 | 0.71 | 22.2 | 20.74 | 20.58 | 15.22 | 0.16 | 5.37 |

Note: TN = Total nitrogen

TKN = Total Kjheda Nitrogen

$NO_3^-$ -N= Nitrate nitrogen

Org-N = Organic nitrogen

**Table B.5** The removal efficiencies of nitrogen constituents at each HRTs and nitrogen loading rate (NLR).

| HRT, hr | NLR , g.Nitrogen/$m^3$-d | $NO_3^-$-N | Org.-N |
|---|---|---|---|
| 12 | 54.82 | 54.00 | 64.69 |
| 16 | 28.88 | 62.50 | 83.07 |
| 20 | 23.10 | 78.41 | 82.80 |
| 24 | 22.90 | 77.46 | 75.84 |

Note: $NO_3^-$ -N= Nitrate nitrogen

Org-N = Organic nitrogen

**Table B.6** The influent and effluent solids, and removal efficiencies at each HRTs

| HRT (h) | Organic Loading Rate ($kgCOD/m^3.d$) | concentration (mg/L) | | | | |
|---|---|---|---|---|---|---|
| | | TS | SS | VSS | TDS | VDS |
| (a) Influent | | | | | | |
| 12 | 2.07 | 755 | 230 | 56 | 525 | 407 |
| 16 | 1.28 | 1075 | 127 | 104 | 948 | 790 |
| 20 | 1.02 | 1075 | 127 | 104 | 948 | 790 |
| 24 | 1.01 | 1046 | 290 | 260 | 756 | 688 |
| (b) Effluent | | | | | | |
| 12 | 2.07 | 312 | 40.0 | 24.9 | 271 | 180 |
| 16 | 1.28 | 319 | 49.0 | 20.0 | 270 | 140 |
| 20 | 1.02 | 297 | 43.0 | 17.0 | 254 | 116 |
| 24 | 1.01 | 183 | 13.33 | 42.0 | 170 | 124 |
| (c) Removal | | | | | | |
| 12 | 2.07 | 58.72 | 82.61 | 55.74 | 48.25 | 55.74 |
| 16 | 1.28 | 70.33 | 61.42 | 80.77 | 71.52 | 82.28 |
| 20 | 1.02 | 72.37 | 66.14 | 83.65 | 73.21 | 85.32 |
| 24 | 1.01 | 82.50 | 95.40 | 83.85 | 77.51 | 81.98 |

**Table B.7** The proportions of nitrogen constituents to total nitrogen in influent and effluent at each HRTs and NLR.

| HRT (h) | NLR , ($g.Nitrogen/m^3$-d) | Influent | | Effluent | | |
|---|---|---|---|---|---|---|
| | | $NO_3^-$-N | Org.-N | $NH_3$ | $NO_3^-$-N | Org.-N |
| 12 | 54.82 | 0.36 | 99.60 | 51.05 | 0.23 | 48.72 |
| 16 | 28.88 | 4.57 | 95.43 | 82.37 | 1.69 | 15.94 |
| 20 | 23.10 | 4.57 | 95.43 | 82.62 | 0.99 | 16.39 |
| 24 | 22.90 | 3.10 | 96.90 | 73.38 | 0.77 | 25.89 |

**Table B.8** In fluent and effluent of phosphorus for each HRTs.

| HRT (h) | PLR $\left(\frac{gP}{m^3 d}\right)$ | Influent , mg/L | | | Effluent, mg/L | | | % removal of org.-P |
|---|---|---|---|---|---|---|---|---|
| | | TP | Ortho-P | Org.-P | TP | Ortho-P | Org.-P | |
| 12 | 3.60 | 1.80 | 1.20 | 0.60 | 1.71 | 1.68 | 0.03 | 95.0 |
| 16 | 1.60 | 1.07 | 0.63 | 0.44 | 0.95 | 0.83 | 0.12 | 72.7 |
| 20 | 1.29 | 1.07 | 0.63 | 0.44 | 0.96 | 0.82 | 0.14 | 68.2 |
| 24 | 0.49 | 0.49 | 0.34 | 0.15 | 0.54 | 0.50 | 0.04 | 73.3 |

**Table B.9** Ratio of ortho-P and organic-P to total phosphorus in influent and effluent.

| HRT (h) | Influent | | Effluent | |
|---|---|---|---|---|
| | Ortho-P | Organic-P | Ortho-P | Organic-P |
| 12 | 66.67 | 33.33 | 98.25 | 1.75 |
| 16 | 58.88 | 41.12 | 87.37 | 12.63 |
| 20 | 58.88 | 41.12 | 85.42 | 14.58 |
| 24 | 69.39 | 30.61 | 92.59 | 7.41 |
| Mean | 63.21 | 36.79 | 92.07 | 7.93 |

**Table B.10** Biogas production rate for each HRT.

| HRT (h) | OLR ($KgCOD/m^3.d$) | Biogas,mL/d | Standard Deviation | $CH_4$, mL/d |
|---|---|---|---|---|
| 12 | 1.01 | 4,100 | 210 | 2,788 |
| 16 | 1.02 | 4,800 | 225 | 3,264 |
| 20 | 1.28 | 6,800 | 365 | 4,624 |
| 24 | 2.07 | 6,400 | 125 | 4,352 |

# Appendix C

## Statistical Analysis for Granule Size at Each Influent Flow Rate

**Table C.1** The percentage of granule size for various flow rates.

| Average granule size (mm) | The percentage of granule size for various flow rate | | | |
|---|---|---|---|---|
| | 16.8 L/day | 20.16 L/day | 25.2 L/day | 33.4 L/day |
| 2.36 | 26.68 | 34.66 | 27.06 | 10.74 |
| 2.00 | 5.38 | 19.20 | 11.18 | 15.14 |
| 1.19 | 63.81 | 35.28 | 44.58 | 42.60 |
| 1.00 | 2.96 | 6.82 | 12.53 | 24.81 |
| 0.84 | 0.79 | 2.18 | 3.37 | 5.19 |
| 0.59 | 0.31 | 0.90 | 0.90 | 1.08 |
| 0.42 | 0.03 | 0.37 | 0.23 | 0.26 |
| 0.25 | 0.02 | 0.59 | 0.15 | 0.18 |
| Mean(mm) | 1,522 | 1,710 | 1,5424 | 1,354 |

**Hypothesis:**

$H_o1$: Mean value of granule size for 16.8L/d flow rate = Mean value of granule size for 20.16 L/d flow rate

$H_a1$: Mean value of granule size for 16.8L/d flow rate ≠ Mean value of granule size for 20.16 L/d flow rate

$H_o2$: Mean value of granule size for 20.16L/d flow rate = Mean value of granule size for 25.2 L/d flow rate

$H_a2$: Mean value of granule size for 20.16L/d flow rate ≠ Mean value of granule size for 25.2 L/d flow rate

$H_o3$: Mean value of granule size for 25.2L/d flow rate = Mean value of granule size for 33.4 L/d flow rate

$H_a3$: Mean value of granule size for 25.2L/d flow rate ≠ Mean value of granule size for 33.4 L/d flow rate

$H_o4$: Mean value of granule size for 16.8L/d flow rate = Mean value of granule size for 33.4 L/d flow rate

$H_a4$: Mean value of granule size for 16.8L/d flow rate ≠ Mean value of granule size for 33.4 L/d flow rate

**Table C.2** The mean comparison test of granule size for each flow rate.

| Z value | H1 (24 – 20 h, HRT) | H2 (20 – 16 h, HRT) | H3 (16 – 12 h, HRT) | H4 (24 – 16 h, HRT) |
|---|---|---|---|---|
| Z caluculated | -0.056 | 0.050 | 0.066 | -0.06 |
| Z table (0.05) | 1.96 | | | |

The calculated Z values for all HRT were not in the critical region, therefore it can be said that all above hypothesises were acceptable. In the other word, the mean value of granule size for 4 flow rates were not different.

# Appendix D

## The Relationship between Biogas Production and COD Removed

**Table D.1** The biogas production and COD removed per day.

| No. of data | COD removed, gCOD/day | Biogas production, L/day |
|---|---|---|
| 1 | 16.97 | 4.64 |
| 2 | 17.44 | 4.64 |
| 3 | 15.56 | 4.64 |
| 4 | 17.92 | 6.47 |
| 5 | 14.85 | 4.30 |
| 6 | 14.85 | 4.30 |
| 7 | 16.03 | 4.73 |
| 8 | 14.35 | 3.60 |
| 9 | 14.35 | 4.20 |
| 10 | 14.14 | 3.30 |
| 11 | 15.09 | 4.20 |
| 12 | 15.33 | 4.20 |
| 13 | 16.27 | 4.20 |
| 14 | 14.11 | 3.60 |
| 15 | 13.64 | 3.40 |
| 16 | 13.64 | 3.40 |
| 17 | 13.18 | 3.32 |
| 18 | 15.18 | 4.63 |
| 19 | 14.94 | 4.52 |
| 20 | 16.58 | 5.48 |
| 21 | 14.78 | 4.20 |
| 22 | 18.97 | 6.72 |
| 23 | 18.68 | 6.37 |
| 24 | 17.64 | 4.79 |

**Table D.2** ANOVA for COD removal (g COD/day) and biogas production (L) per day.

| Model | Sum of squares | df | Mean square | F | Sig. |
|---|---|---|---|---|---|
| Regression | 16.974 | 1 | 16.974 | 95.562 | 0.000 |
| Residual | 3.908 | 22 | 0.178 | | |
| Total | 20.881 | 23 | | | |

# Appendix E

## Determination of Kinetic Coefficients

**Determination of kinetic coefficients**

The parameters of Y, k, $K_s$ and $k_d$ must be estimated for using biological wastewater kinetic model. To determine the above coefficients values, the results obtained from pilot scale were used.

In this study, the five different θc were used to determine the coefficient values. During steady-state condition, collected data for each $\theta_c$ (mean, values) were used to determine Q, $S_o$, S, X and $r_{su}$. The following equation were used to determine $r_{su}$.

$$r_{su} = \frac{-kXS}{(K_s + S)} \qquad \text{(E-1)}$$

Where

$r_{su}$. = Substrate utilization rate, mass/unit volume.time

X = Concentration of microorganism

S = Concentration of growth-limiting substrate in solution, mass/unit.volume

Ks = half-velocity constant

k = maximum rate of substrate utilization per unit mass of microorganisms

From equation (4-4)

$$r_{su} = \frac{-kXS}{(K_s + S)} = \frac{(S_0 - S)}{\theta} \qquad \text{(E-2)}$$

Dividing equation (4-5) by X, yields

$$\frac{kS}{(K_s + S)} = \frac{S_0 - S}{\theta} \qquad \text{(E-3)}$$

Linearlization equation (4-6) by taking its inverse,

$$\frac{(K_s + S)}{kS} = \frac{\theta}{S_0 - S}$$

$$\frac{\theta X}{S_0 - S} = \frac{K_s}{kS} + \frac{1}{k} \qquad \text{(E-4)}$$

The values of $K_s$ and k were determined by plotting $\frac{\theta X}{S_0 - S}$ versus $\frac{1}{S}$ as illustrated in Figure E-1 whereas the values of $Y$ and $k_d$ were determined by plotting $\frac{1}{\theta_c}$ versus $\frac{-r_{su}}{X}$ as presented in Figure E-2 and equation below.

$$\frac{1}{\theta_c} = \frac{-Yr_{su}}{X} \qquad \text{(E-5)}$$

The μm coefficient was determined by following equation and the relationship of μ and substrate is shown in Figure E-3.

$$\mu_m = kY \qquad \text{(E-6)}$$

The values of kinetic parameters are shown in Table E-1.

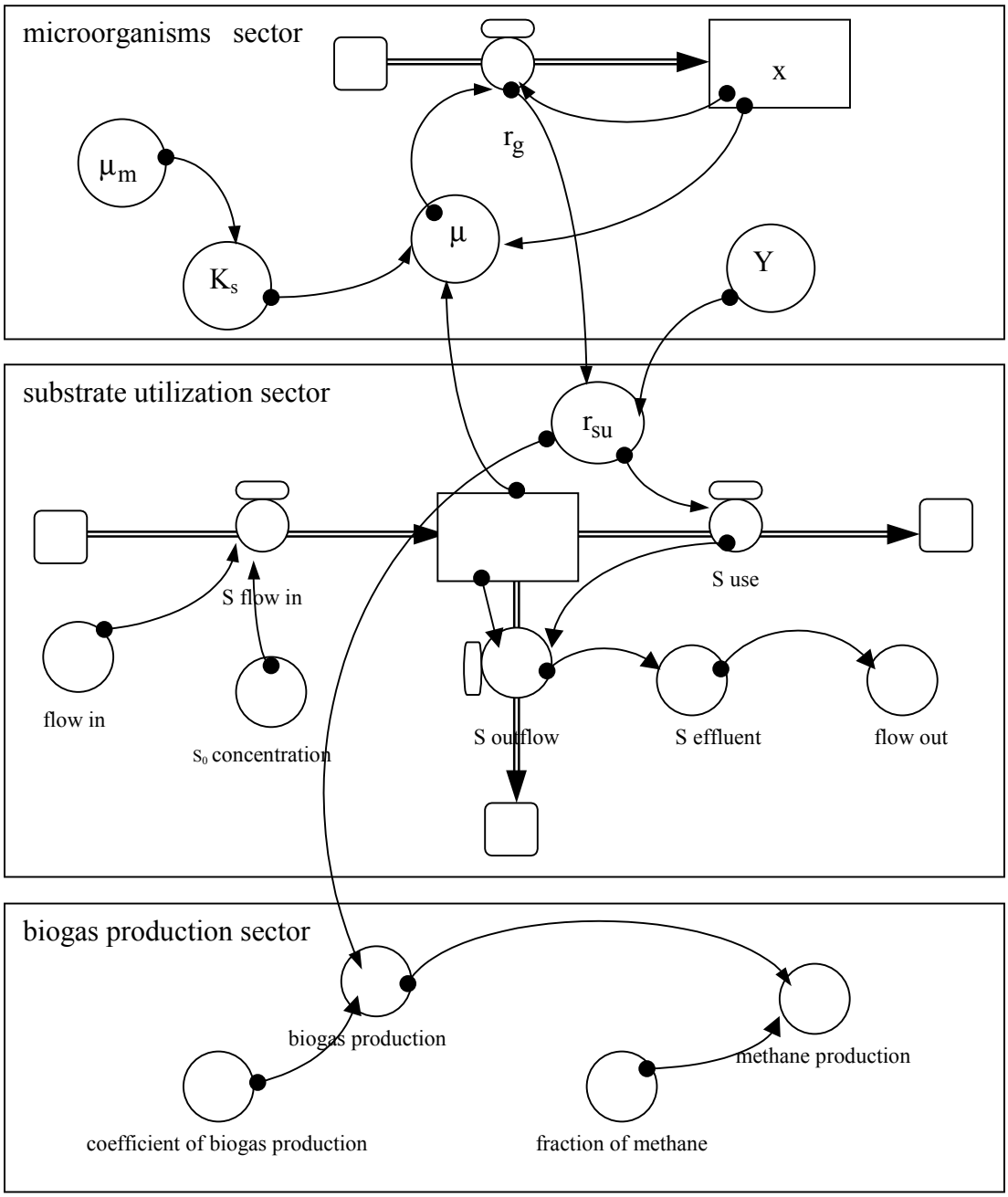
**Table E.1** The kinetics parameters obtained from this research.

| Kinetic parameters | Value |
|---|---|
| k | 13 |
| $K_s$ | 14.73 |
| Y | 0.189 |

# Appendix F

## The Flow Diagram and Equation of Constructed Model

## in the STELLA Software Format




**Equation**

Biogas production sector

biogas_formation = rsu*coefficient_of_biogas_production/1000

coefficient_of_biogas_production = 0.375

fraction_of_Methane = 0.68

Methane_production = (biogas_formation*fraction_of_Methane)

Microorganisms sector

X(t) = X(t - dt) + (rg) * dt

INIT X = 103*8

INFLOWS:

rg = u*X

Ks = 14.73

μ = μmax*S/(Ks*X+S)

μmax = 2.48

Y = 0.19

Substrate utilization sector

s(t) = s(t - dt) + (s_flowin - s_outflow - s_use) * dt

INIT s = 0

INFLOWS:

s_flowin = flow_in*S0_Conc

OUTFLOWS:

s_outflow = s-s_use

s_use = rsu

flow_in = 16.8

flow_out = 16.8

rsu = rg/Y

S0_Conc = 1007

seff = s_outflow/flow_out

Not in a sector

# Appendix G

## The Relationship of Actual Biogas Production and Estimation by Using Constructed Model

**Table G.1** Actual biogas production and estimation.

| Actual (mL/d) | Estimation (mL/d) | $V = \frac{\Sigma(y_i x_i)^2}{\Sigma X_i^2}$ |
|---|---|---|
| 6369 | 6230 | 0.000498 |
| 6720 | 6320 | 0.004006 |
| 6473 | 5980 | 0.006797 |
| 5483 | 5530 | 7.22E-05 |
| 4627 | 5060 | 0.007323 |
| 6347 | 5540 | 0.021219 |
| Sum | | 0.039914 |

**Table G.2** ANOVA of linear regression analysis for monitored biogas production and estimation.

| Model | Sum of Squares | df | Mean square | F | Sig. |
|---|---|---|---|---|---|
| Regression | 2418693.2 | 1 | 2418696.226 | 13.210 | 0.022 |
| Residual | 732360.61 | 4 | 183090.152 | | |
| Total | 3151056.8 | 5 | | | |

**Table G.3** Data for model calibration and validation

| No. | Run 1 (Calibration) | | Run 2 & 3 (Validation) | |
|---|---|---|---|---|
| | actual COD | estimated COD | actual COD | estimated COD |
| 1 | 126.3 | 123.06 | 87.67 | 89.7 |
| 2 | 140.3 | 139.47 | 93.7 | 95.88 |
| 3 | 112.2 | 114.85 | 70.6 | 82 |
| 4 | 113.9 | 108.23 | 87 | 96.3 |
| 5 | 96.3 | 104.58 | 85 | 96.3 |
| 6 | 126.3 | 139.47 | 87.4 | 89.7 |
| 7 | 98.2 | 114.85 | 93.2 | 89.7 |
| 8 | 110.08 | 104.58 | 95 | 102.5 |
| 9 | | | 70.6 | 82.5 |
| 10 | | | 91 | 96.29 |

**Table G.4** ANOVA result for model calibration.

| Model | Sum of Squares | df | Mean square | F | Sig. |
|---|---|---|---|---|---|
| Regression | 970.664 | 1 | 970.664 | 12.968 | 0.011 |
| Residual | 449.114 | 6 | 74.852 | | |
| Total | 1419.778 | 7 | | | |

**Table G.5** ANOVA result for model calibration.

| Model | Sum of Squares | df | Mean square | F | Sig. |
|---|---|---|---|---|---|
| Regression | 255.60 | 1 | 255.600 | 15.593 | 0.004 |
| Residual | 131.13 | 8 | 16.392 | | |
| Total | 386.73 | 9 | | | |

# Biography

Mr. Kraichat Tantrakarnapa was born on May 18, 1964, in Lampang, North of Thailand. After the secondary school at Khelangnakorn school, Lampang, studied the undergraduate program in Statistics at Chiangmai University. With the Bachelor degree, he worked as computer supervisor at Office of Rector, Mahidol University, and continued the Master program in Technology of Environmental Management, Mahidol University and worked as research assistant at Faculty of Environment and Resources Studies after finishing the program. Subsequently, he worked as Environmental Specialist at Team Consulting Engineering and Management Co.,Ltd. Bangkok. He pursued the Ph.D. program in Environmental Engineering at Suranaree University of Technology, in the second trimester of 1999. Up to the present time, he has been working as a lecturer at Department of Environmental Health Sciences, Faculty of Public Health, Mahidol University since 2001.