ANAEROBIC BAFFLED REACTOR (ABR): OPTIMUM SRT/HRT RATIO AND ORGANIC LOADING RATE FOR TREATMENT OF CARBOHYDRATE-PROTEIN WASTEWATER

APAPORN RUCHIRASED

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE
(ENVIRONMENTAL TECHNOLOGY)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2007

Thesis

Entitled

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Apaporn Ruchirased.

Miss Apaporn Ruchirased
Candidate

S.C.vanich

Lect. Sopa Chinwetkitvanich,
Ph.D. (Environmental Engineering)
Major-Advisor

P. R.

Assoc. Prof. Prayoon Fongsatitkul,
Ph.D. (Environmental Engineering)
Co-Advisor

Prof. M.R. Jisnuson Svasti,
Ph.D.
Dean
Faculty of Graduate Studies

P. R.

Assoc. Prof. Prayoon Fongsatitkul,
Ph.D. (Environmental Engineering)
Chair
Master of Science Programme in
Environmental Technology
Faculty of Public Health

Thesis

Entitled

ANAEROBIC BAFFLED REACTOR (ABR): OPTIMUM SRT/HRT RATIO AND ORGANIC LOADING RATE FOR TREATMENT OF CARBOHYDRATE-PROTEIN WASTEWATER

was submitted to the Faculty of Graduate Studies, Mahidol University

For the degree of Master of Science (Environmental Technology)

on

May 9, 2007

Apaporn Ruchirased

Miss Apaporn Ruchirased
Candidate

C. Phornpimolthape

Assoc. Prof. Chaovayut Phornpimolthape,
M.S.I.E. (Operations Reasearch)
Chair

Lect. Somkiet Techkarnjanaruk,
Ph.D. (Molecular Microbiology)
Member

S.C.vanich

Lect. Sopa Chinwetkitvanich,
Ph.D. (Environmental Engineering)
Member

Asst. Prof. Bunyarit Panyapinyopol,
Ph.D. (Environmental Management)
Member

P. P.

Assoc. Prof. Prayoon Fongsatitkul,
Ph.D. (Environmental Engineering)
Member

Prof. M.R. Jisnuson Svasti,
Ph.D.
Dean
Faculty of Graduate Studies
Mahidol University

Phitaya Ch, M.D.

Assoc. Prof. Phitaya Charupoonphol,
M.D., Dip. Thai Board of Epidemiology
Dean
Faculty of Public Health
Mahidol University

ACKNOWLEDGEMENTS

I would like to particularly gratitude and appreciation to Lect. Dr. Sopa Chinwetkitvanich for her advice, guidance, and financial support throughout this work, critical reading and kindly suggestion improvements of the manuscript. I also would like to thank Assoc. Prof. Dr. Prayoon Fongsatitkul, Assoc. Prof. Chaovayut Phornpimolthape, and Asst. Prof. Bunyarit Panyapinyopol for their correction the document and constructive comments.

I would like to sincere gratitude to Dr. Somkiet Techkarnjanaruk, National Center for Genetic Engineering and Biotechnology (BIOTEC), of Pilot Plant Development and Training Institute, King Mongkut's University of Technology, Thonburi for his practice, guidance, support for FISH technique, and chiefly for his trust in me. I also thank Mr. Wiwat Jupraputtasri, Miss Nimaradee Boonapatcharoen, and the member of Biogas and Gene laboratory for their kind assistance with FISH procedure, equipment, and friendliness.

I also express my gratitude to the staff of Department of Sanitary Engineering, Faculty of Public Health, Mahidol University for their co-operation and generous assistance. I especially like to gratefully thank to my friend, my senior and my junior of "PHSE" for their encouragement, all assistant, merriment, and laugh.

This research work is supported by the grant from the Post-Graduate Education, Training and Research Program in Environmental Science, Technology and Management under Higher Education Development Project of the Commission on Higher Education, Ministry of Education.

Finally, I would like to appreciation and thank to my family for entirely care moral and financial support, and unconditional love on me.

Apaporn Ruchirased

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APAPORN RUCHIRASED 4736276 PHET/M
M.Sc. (ENVIRONMENTAL TECHNOLOGY)

THESIS ADVISORS: SOPA CHINWETKITVANICH, Ph.D. (Env. Eng.)
PRAYOON FONGSATITKUL, Ph.D. (Env. Eng.)

ABSTRACT

This study was divided into two parts; the first was to investigate the effect of compartment numbers on SRT and SRT/HRT ratio, and the second was to determine an optimum organic loading rate (OLR) of anaerobic baffled reactor (ABR) treated carbohydrate-protein wastewater. The first part were done with three 10-liters laboratory scale ABR with different three compartment numbers i.e. 3, 6, and 8 compartments (named as 3C, 6C, and 8C experiments, respectively) and operated to the purpose HRT of 24 hrs with OLR of 4 g COD/l-d (OLR4). The reactor with appropriate compartment numbers from this part was selected for further investigation of the optimum OLR. Three OLR, i.e. 4, 8, 12, and 16 g COD/l-d (named as OLR4, OLR8, OLR12, and OLR16, respectively) were investigated with the same operating HRT as in the first part.

The results from the first part evidently showed that the compartmentalized structure of ABR helped retard sludge washout rate. High numbers of compartment lowered sludge washout, consequently, sludge could be maintained in the reactor for longer period. Also, the COD removal efficiencies increased with high compartment numbers; i.e. the 3C-OLR4, 6C-OLR4, and 8C-OLR4 experiments achieved 74, 78, and 83% of COD removal efficiencies with SRT/HRT ratios of 35, 74, and 134 d/d, respectively. Hence, based on this study the optimum SRT/HRT ratio was achieved from the eight-compartment ABR with SRT/HRT ratio of 134 d/d.

In the second part, the eight-compartment ABR was selected to determine the optimum OLR. The results obtained from the experiments showed that the COD removal efficiency increased from 83 to 96% as the OLR increased from 4 to 8 g COD/l-d. When OLR was increased to 12 g COD/l-d, the COD removal efficiency was about 88%. Unfortunately, the system of 16 g COD/l-d OLR became sour and was eventually failed. In conclusion, the OLR as high as 12 g COD/l-d applied to an ABR could still achieve high COD removal efficiency of above 80%, particularly when treating this such a low solid content wastewater. However, the OLR of 16 g COD/l-d was not concluded to be overload for ABR system. In addition, studying of the microbial populations by FISH technique proved the assumption of microbial phase separation in ABR system.

KEY WORDS: ANAEROBIC BAFFLED REACTOR/ COMPARTMENTALIZATION/ CARBOHYDRATE-PROTEIN WASTEWATER/ SRT/HRT RATIO/ FISH TECHNIQUE

98 pp.

ถังปฏิกรณ์แผ่นกั้นไร้อากาศ: อัตราส่วนอายุสลัดจ์ต่อเวลากักพักน้ำและอัตราภาระอินทรีย์ที่เหมาะสมในการบำบัดน้ำเสียคาร์โบไฮเดรตโปรตีน (ANAEROBIC BAFFLED REACTOR (ABR): OPTIMUM SRT/HRT RATIO AND ORGANIC LOADING RATE FOR TREATMENT OF CARBOHYDRATE-PROTEIN WASTEWATER)

อาภาพร รุจิระเศรษฐ 4736276 PHET/M
วท.ม. (เทคโนโลยีสิ่งแวดล้อม)

คณะกรรมการควบคุมวิทยานิพนธ์: โสภา ชินเวชกิจวานิชย์ Ph.D. (Env. Eng.), ประยูร ฟองสถิตย์กุล Ph.D. (Env. Eng.)

บทคัดย่อ

การศึกษานี้แบ่งออกเป็น 2 ส่วน ส่วนแรกเป็นการศึกษาผลกระทบของจำนวนช่องที่มีต่ออัตราส่วนอายุสลัดจ์ต่อเวลากักพักน้ำของระบบแผ่นกั้นไร้อากาศ และส่วนที่สองเป็นการศึกษาอัตราภาระอินทรีย์ที่เหมาะสมในการบำบัดน้ำเสียคาร์โบไฮเดรต-โปรตีน การทดลองในส่วนแรกใช้ถังปฏิกรณ์แผ่นกั้นไร้อากาศจำนวน 3 ถัง ซึ่งมีปริมาตรประสิทธิผล 10 ลิตรในแต่ละถังปฏิกรณ์แบ่งเป็นช่องจำนวน 3, 6 และ 8 ช่อง (3C, 6C และ 8C ตามลำดับ) ทำการทดลองโดยใช้เวลากักพักน้ำ 24 ชั่วโมง และอัตราภาระอินทรีย์ 4 กรัมซีโอดี/ลิตร-วัน ถังปฏิกรณ์ที่มีจำนวนช่องที่เหมาะสม โดยพิจารณาจากประสิทธิภาพการกำจัดซีโอดีของระบบจะถูกนำไปใช้เพื่อทดลองหาอัตราภาระอินทรีย์ที่เหมาะสม อัตราภาระบรรทุกสารอินทรีย์ที่ทำการศึกษาคือ 4, 8, 12 และ 16 กรัมซีโอดี/ลิตร-วัน (OLR4, OLR8, OLR12 และ OLR16 ตามลำดับ) ทำการทดลองที่เวลากักพักน้ำ 24 ชั่วโมงเท่ากับการทดลองในส่วนแรก

ผลการทดลองในส่วนแรกแสดงให้เห็นอย่างชัดเจนว่าโครงสร้างที่แบ่งเป็นช่องของถังปฏิกรณ์แผ่นกั้นไร้อากาศสามารถช่วยชะลอการหลุดของสลัดจ์ออกจากระบบได้ จำนวนช่องที่มากกว่าส่งผลให้อัตราการหลุดของสลัดจ์น้อยลง ทำให้สามารถเก็บรักษาสลัดจ์ไว้ในระบบได้นานขึ้น และนอกจากนี้ยังมีประสิทธิภาพการกำจัดซีโอดีมากกว่าอีกด้วย โดยพบว่าประสิทธิภาพในการกำจัดซีโอดีในชุดการทดลอง 3C-OLR4, 6C-OLR4 และ 8C-OLR4 คือ 74, 78 และ 83% ตามลำดับ และอัตราส่วนอายุสลัดจ์ต่อเวลากักพักน้ำเท่ากับ 35, 74 และ 134 วัน/วัน ตามลำดับ ผลการศึกษาในส่วนนี้พบว่าถังปฏิกรณ์ที่มี 8 ช่อง มีอัตราส่วนอายุสลัดจ์ต่อเวลากักพักน้ำที่เหมาะสมคือ 134 วัน/วัน

ในส่วนที่สองใช้ถังปฏิกรณ์แผ่นกั้นไร้อากาศที่มีจำนวน 8 ช่อง มาศึกษาอัตราภาระสารอินทรีย์ที่เหมาะสม จากการทดลองจะเห็นว่าประสิทธิภาพการกำจัดซีโอดีเพิ่มขึ้นจากร้อยละ 83 เป็นร้อยละ 96 เมื่ออัตราภาระอินทรีย์เพิ่มขึ้นจาก 4 เป็น 8 กรัมซีโอดี/ลิตร-วัน เมื่อเพิ่มอัตราภาระอินทรีย์เป็น 12 กรัมซีโอดี/ลิตร-วัน ประสิทธิภาพการกำจัดซีโอดีอยู่ที่ประมาณร้อยละ 88 และที่อัตราภาระบรรทุกสารอินทรีย์ 16 กรัมซีโอดี/ลิตร-วัน ระบบเริ่มมีกลิ่นเหม็นเปรี้ยวและล้มเหลวในที่สุด จึงสรุปได้ว่าที่อัตราภาระบรรทุกสารอินทรีย์ที่น้อยกว่า 12 กรัมซีโอดี/ลิตร-วัน ถังปฏิกรณ์แผ่นกั้นไร้อากาศสามารถเดินระบบได้อย่างมีประสิทธิภาพ โดยมีประสิทธิภาพในการกำจัดซีโอดีมากกว่าร้อยละ 80 สำหรับการบำบัดน้ำเสียที่มีปริมาณของแข็งแขวนลอยในน้ำต่ำ อย่างไรก็ตามยังไม่สามารถสรุปได้อย่างแน่ชัดว่าที่อัตราภาระอินทรีย์ 16 กรัมซีโอดี/ลิตร-วัน นั้นเป็นภาระสารอินทรีย์ที่สูงเกินกว่าถังปฏิกรณ์แผ่นกั้นไร้อากาศจะสามารถบำบัดได้ สำหรับการศึกษาประชากรแบคทีเรียโดยเทคนิค FISH ได้พิสูจน์สมมติฐานที่ว่าถังปฏิกรณ์แผ่นกั้นไร้อากาศสามารถแยกกลุ่มจุลินทรีย์ในการเดินระบบได้

98 หน้า

CONTENTS

CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

Page

FIGURE

LIST OF FIGURES (continued)

Page

FIGURE

LIST OF FIGURES (continued)

Page

FIGURE

LIST OF ABBREVIATIONS

ABR	Anaerobic Baffled Reactor
Alk	Alkalinity
CSTR	Continuously Stirred Tank Reactor
Eff	Effluent
FISH	Fluorescence *in situ* Hybridization
HRT	Hydraulic Retention Time
Inf	Influent
MLSS	Mix Liquor Suspended Solid
MLVSS	Mix Liquor Volatile Suspended Solid
OLR	Organic Loading Rate
ORP	Oxidation Reduction Potential
sCOD	Soluble COD
SRT	Solid Retention Time
tCOD	Total COD
TSS	Total Suspended Solid
UASB	Up-flow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acid
VSS	Volatile Suspended Solid
°C	Degree Celsius

CHAPTER I
INTRODUCTION

1.1 Rationales and Justifications

Over the past decades, anaerobic fermentation and oxidation processes have been widely used for treatment of wastewater. Their several advantages over other aerobic processes of wastewater treatment have been aware such as energy saving, less biomass production, low construction and operation cost, high removal efficiency and energy as a by-product.

Nonetheless, anaerobic process usually has a problem of maintaining biomass in the reactor. In a conventional completely mixed or plug flow digester, solid retention time (SRT) would be usually equal to hydraulic retention time (HRT). Therefore, in order to keep the biomass within the reactor as long as possible, high volume of reactor is necessary. Subsequently, several modifications to solve this problem were attempted, such as returning the biomass into the reactor, using a filter for trapping the biomass or even packing some media in the reactor. However, the cost of filter and packing material becomes the disadvantage. In addition, the problems of clogging, more pumping power required or the need of the packing level control and wasting with ingrowths were much concerned.

An anaerobic baffled reactor (ABR) is a high-rate anaerobic reactor using a series of vertical baffles to direct the flow upward and downward from inlet to outlet. It can be implied that the ABR reactor acts like a series of UASB (upflow anaerobic sludge blanket), but no need for sludge granulation and gas-solids separation device. ABR has a higher resistance of both hydraulic and organic shock loads than some other anaerobic processes. ABR can be designed to improve biomass retention in reactor,

resulting in a longer SRT (Nachaiyasit and Stuckey, 1997b; 1997c) without need of packing media or a solid-settling chamber or sludge granulation.

The compartmentalized structure in ABR is an important key of retaining biomass within the reactor. The more compartments in a reactor, the better biomass retention is. Also, this structure is helpful in separating acidogenic and methanogenic phases, which will enhance stability and higher organic loading rate (OLR) of the anaerobic process, as well as, increase the overall removal efficiency with shorter HRT (Demirer and Chen, 2005). Several studies have found that the ABR could be operated with HRT less than 1 day (Grobicki and Stuckey, 1991; Nachaiyasit and Stuckey, 1997b; 1997c; Bell and Buckley, 2003). The successful operation of ABR in treating of domestic, industrial and agricultural wastewater with the removal efficiency higher than 90% were reported (Dama et al., 2002; Foxon et al., 2004; Bell et al., 2000; Grover et al., 1999; Boopathy et al., 1998; Boopathy and Sievers, 1991).

Although ABR has been developed for over twenty years, the knowledge in designing such a reactor has still not been clarified. The most advantage of ABR is its SRT and HRT can be operated separately. Therefore, SRT can be increased over HRT several times. This will benefit in a smaller size of reactor while still achieving high biomass concentration and consequently high efficiency. The SRT/HRT ratio is an important parameter to compare the effectiveness of ABR technically and economically. The aim of this study is to investigate an appropriate SRT/HRT ratio and OLR of ABR treating carbohydrate-protein wastewater.

1.2 Research Objectives

1.2.1 General Objective

To investigate an optimum SRT/HRT ratio and organic loading rate of the anaerobic baffled reactor treating carbohydrate-protein wastewater.

1.2.2 Specific Objectives

1.2.2.1 To investigate the effect of compartment numbers on SRT and SRT/HRT ratio of the anaerobic baffled reactor treating carbohydrate-protein wastewater.

1.2.2.2 To investigate the optimum organic loading rate of the anaerobic baffled reactor treating carbohydrate-protein wastewater.

1.3 Research Hypotheses

1.3.1 SRT and SRT/HRT ratio will increase with increasing of numbers of compartment.

1.3.2 COD removal efficiency will increase with increasing of SRT/HRT ratio.

1.4 Research Variables

1.4.1 Part I: Effect of Compartment Numbers on SRT and SRT/HRT Ratio

1.4.1.1 Independent Variables

- Numbers of compartment (3, 6, and 8 compartments)

1.4.1.2 Dependent Variables

- Solid retention time (SRT)
- Chemical oxygen demand (COD)

1.4.1.3 Control Variables

- Effective volume of reactor (10 liters)
- Influent COD concentration (4,000 mg/l) } OLR_1
- Hydraulic retention time (HRT) (24 hrs) } OLR_1
- Inoculated MLSS concentration

1.4.2 Part II: Optimum Organic Loading Rate

1.4.2.1 Independent Variables

- Organic loading rates (OLR_2, OLR_3, and OLR_4)

1.4.2.2 Dependent Variables

- Chemical oxygen demand (COD)
- Solid retention time (SRT)

1.4.2.3 Control Variables

- Effective volume of reactor (10 liters)
- Hydraulic retention time (HRT) (24 hrs)
- Numbers of compartment
- Inoculated MLSS concentration

1.5 Scope of Study

1.5.1 Wastewater used in this study was the synthetic carbohydrate-protein wastewater.

1.5.2 Numbers of compartment in the ABR using in this study were 3, 6, and 8 compartments.

1.5.3 Influent COD was controlled in the range of 4,000-16,000 mg COD/l.

1.6 Limitation of the Study

This experimental lab–scale apparatus was installed and operated at the laboratory without a temperature control.

1.7 Definition of Keywords

1.7.1. Anaerobic Baffled Reactor (ABR): The Compartmentalizing of anaerobic reactor, with a series of vertical baffles in the reactor to force and direct the

flow of wastewater to flow downward and upward (or through) the baffles as it passes from the inlet to outlet.

1.7.2 Solid Retention Time (SRT): The average residence time of suspended solids in a biological treatment system. The calculation in this study is follows equation 1.1 (Udomsinrot, 2000).

$$\text{SRT (d)} = \frac{\text{Mass in reactor (g)}}{\text{Sludge washout rate (g/d)}} \qquad (1.1)$$

1.7.3 Hydraulic Retention Time (HRT): The average residence time of hydraulic flow in a treatment system. The calculation of HRT is follows equation 1.2.

$$\text{HRT (d)} = \frac{\text{Volume of reactor (l)}}{\text{Flow rate (l/d)}} \qquad (1.2)$$

1.7.4 Mixed Liquor Suspended Solids (MLSS): The concentration of suspended solids in mixed liquor, expressed in milligrams per liter (mg/l).

1.7.5 Organic Loading Rate (OLR): The amount of organic material, typically measured as COD, applied to a given treatment process, which calculated by equation 1.2.

$$\text{OLR(g/l - d)} = \frac{\text{COD (g/l)}}{\text{HRT (d)}} \qquad (1.3)$$

1.7.6 Fluorescence *in situ* Hybridization (FISH): A molecular technique used to detect microorganism.

1.8 Conceptual Framework

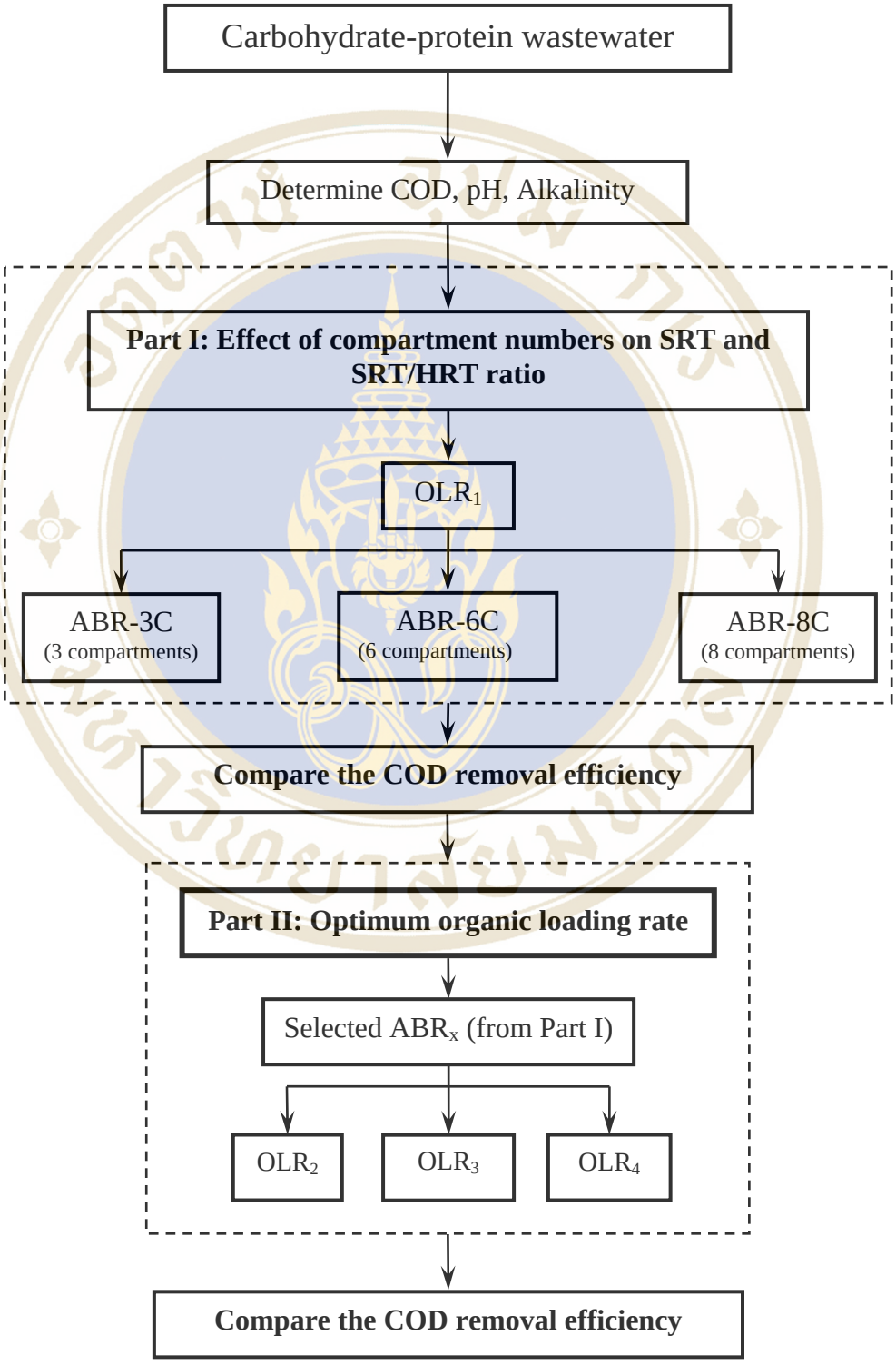


Figure 1.1 Conceptual framework

CHAPTER II
LITERATURE REVIEWS

2.1 Anaerobic Processes

Anaerobic processes have been used in wastewater treatment systems for more than a century. In the early period, they are attractive processes, especially for solids stabilization and high strength wastewater treatment. To date, applications for dilute wastewater treatment have also been demonstrated and are becoming more common (Metcalf & Eddy, 2004). The term of anaerobic condition refers to a series of microbiological process that convert the majority of organic compound to methane (CH_4) and carbon dioxide (CO_2) without the existence of dissolved oxygen and nitrate-N.

2.1.1 Process Fundamental

The overall anaerobic oxidation of wastewater is usually described by three major steps: hydrolysis, fermentation (also known as acidogenesis), and methanogenesis. Nevertheless, for the greater understanding, the anaerobic oxidation is also divided into six reactions, which are shown consecutively in Figure 2.1, namely:

1. Hydrolysis of complex organic materials into smaller molecules such as proteins, carbohydrate and lipids hydrolyzed into amino acids, sugars and fatty acids, respectively. The reactions are catalyzed by extracellular enzymes produced by microorganisms, e.g. cellulases, amylases, and proteases.

2. Fermentation of amino acids and sugars, which organic compounds serve as both electron donors and acceptors. The products of this reaction mostly are volatile fatty acids; therefore, the reaction can be also called as acidogenesis. The important products from this reaction are acetic acid and H_2 released from the dehydrogenation of pyruvate as they are the direct precursor for methane production. In addition, intermediary degradable products like propionic and butyric acids are also the products from this sort of reaction.

3. Anaerobic oxidation of long chain fatty acid and alcohols to acetic acid and H_2. The amount of produced H_2 from this reaction is usually higher than from the above reaction because of the transfer of electrons from reduced carriers directly to hydrogen ions. The importance of produced H_2 is its inhibition to this anaerobic oxidation itself when high partial pressure of H_2 occurs.

4. Anaerobic oxidation of intermediary products such as volatile acids, (except acetic acid), to acetic acid. Similar to the reaction 3, the produced H_2 will be released during the oxidation and can inhibit the reaction under the occurrence of high partial pressure of H_2.

5. Conversion of acetic acid to methane gas. The bacteria concerned in this reaction are called aceticlastic methanogens, which split acetic acid into methane and carbon dioxide.

6. Conversion of hydrogen to methane gas. The bacteria so called H_2-oxidizing methanogens are responsible for the conversion to methane by reducing carbon dioxide and using H_2 as an electron acceptor. These methanogens are obligately linked to the ones in the reactions 3 and 4 because the required substrate, H_2. Likewise, the above bacteria are also obligately linked to the methanogens as their continuous utilization of H_2 will keep low partial pressure of H_2 and allow the oxidations go on. Such a relationship between the two bacterial groups is called “syntrophy”.

2.1.2 Process Microbiology

Anaerobic processes are complex ecosystems comprising diverse microorganisms, which work together in a coordinated manner to convert organic substance to methane (CH_4) and carbon dioxide (CO_2). They use organic substance as a carbon source for thriving, in which there is no substantial amount of molecular oxygen for anaerobic degradation of organic matter (Reynolds and Richards 1996; Bitton, 2005). The overall reaction is shown in equation 2.1 (Bitton, 2005).

$$\text{Organic substance} \rightarrow CH_4 + CO_2 + H_2 + NH_3 + H_2S \qquad (2.1)$$

The microbial communities in anaerobic processes are divided into four categories, that is;

- *Hydrolytic bacteria:* Break down complex organic molecule (e.g., proteins, cellulose, lignin, lipids) into soluble monomer molecules such as amino acids, glucose, fatty acids, and glycerol.

- *Fermentative acidogenic bacteria:* Convert soluble monomer molecules such as amino acids, sugars, fatty acids to volatile fatty acids (e.g., acetic, propionic, butyric), alcohols and ketones, CO_2 and H_2.

- *Acetogenic bacteria:* Convert volatile fatty acids (exclude acetic acid) and alcohols into acetate, CO_2 and H_2, which are further utilized by methanogens.

- *Methanogens:* Use a limited number of substrates, e.g., acetate, H_2, CO_2, formate, methanol, and methylanimes. All of these substrates can be directly converted to CH_4.

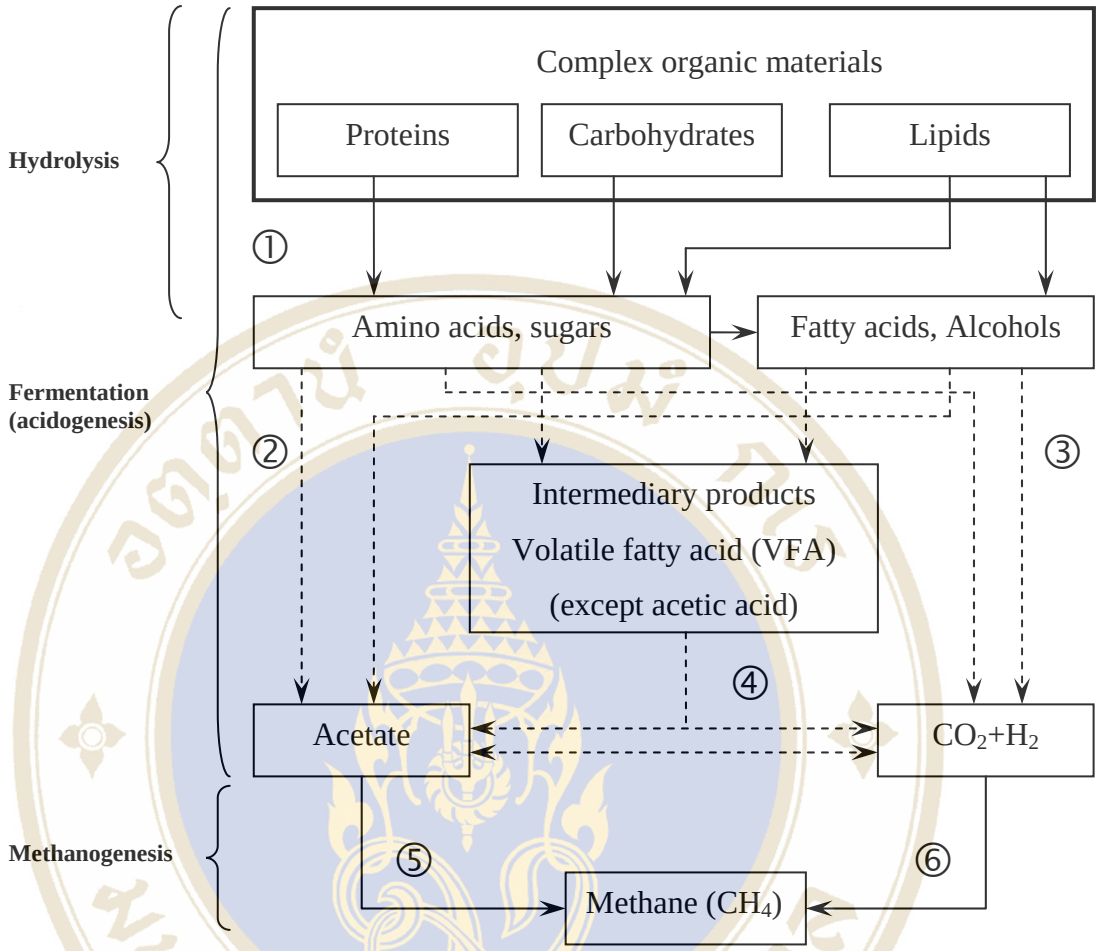


Figure 2.1 Conversion processes of anaerobic fermentation and oxidation (Adapted from Gujer and Zehnder, 1983; Anderson et al., 2003).

The functioning of these microbes is involved in the transformation of complex materials into simple molecules as shown in Figure 2.2.

2.1.3 Factors Affecting Anaerobic Processes

Appropriate environments for anaerobic microbial activities are an important factor in the effective removal of pollutants in anaerobic wastewater treatment. The process is affected by several factors as follow

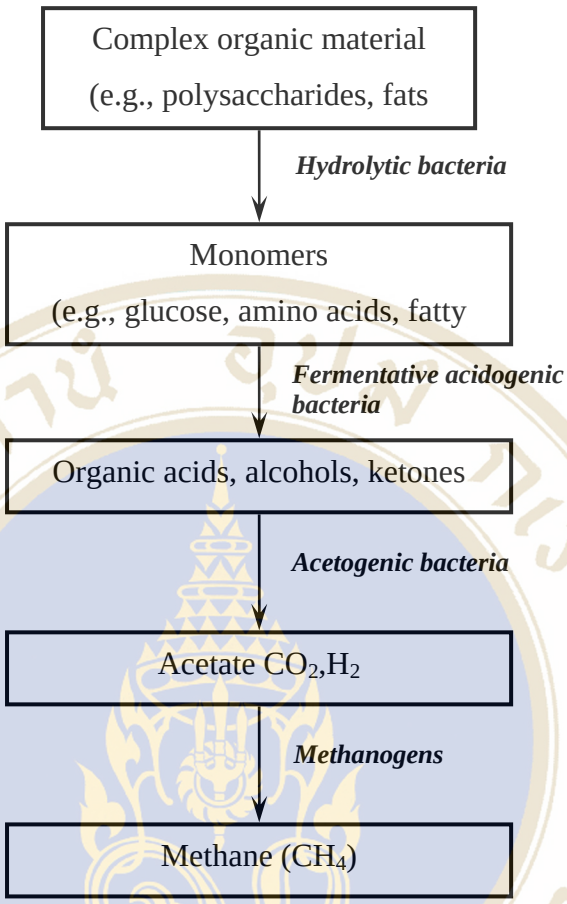


Figure 2.2 Metabolic bacterial group involved in anaerobic digestion of wastes (Bitton, 2005).

2.1.3.1 Nutrients: Nutrients can be divided into two groups; the *macronutrients* and *micronutrients*. The *macronutrients* are such as organic substrates, a source of carbon and energy to sustain growth and to carry out biochemical transformation. A number of anaerobic microorganisms require some essential *micronutrients* for bacterial metabolism, growth, activity, and consequently stability of process. Although, several nutrients are necessary for anaerobic microbes, but some nutrients can become toxic when present in high concentration (Anderson et al., 2003). Anaerobic processes produce less biomass than aerobic ones, hence, they require less nitrogen and phosphorus for biomass anabolism. The COD:N ratio of 400:7 at high loading rates (0.8-1.2 g COD/g VSS-d) and phosphorus requirement is

about 2.2% of N are recommended (Singh et al., 1999). The supplementary requirement of micronutrients for anaerobic processes as shown in Table 2.1.

Table 2.1 Micronutrients requirement on anaerobic digestion and fermentation processes.

Nutrients	Required concentration (mg/l)	Purpose
Ca	100-200	Improving ability of sludge flocculation
Mg	75-150	Stimulating growth and shorten the generation time.
Na	100-200	Increasing in activity
K	200-400	Increasing in activity
SO_4	0.1-10	Sulfur source of cell synthesis

Sources: Singh et al., 1999; Anderson et al., 2003.

2.1.3.2 Temperature: Reactor temperatures of 25-35°C are generally preferred to support more optimal biological reaction rate and to provide more process stability (Metcalf & Eddy, 2004).

2.1.3.3 Hydraulic retention time (HRT): HRT is affecting both reactor volume and biomass, the less HRT, the smaller of reactor volume. Nonetheless the too low HRT can cause sludge washing out.

2.1.3.4 Solid retention time (SRT): SRT is a fundamental design and operating parameter for all anaerobic processes. According to the longer double time required for anaerobic bacteria, SRT values greater than 20 days are needed for general anaerobic processes at 30°C in order to obtain effective treatment performance. Moreover, the higher SRT values should be considered when the processes operated at lower temperature (Metcalf & Eddy, 2004).

2.1.3.5 pH: Neutral pH in anaerobic reactor should be maintained to ensure efficient methanogenic digestion can proceed. Most methanogens function optimally at a pH range of 6.7-7.4 as the failure of the process may occur if the pH is close to 6.0 (Bitton, 2005).

2.1.2.6 Alkalinity: Alkalinity is a measure of the buffering or acid-neutralizing capacity of the digester. Generally, the alkalinity concentration above 2,500 mg/l as $CaCO_3$ is recommended (Fannin, 1987).

2.1.2.7 Oxidation-reduction potential (ORP): During strict anaerobic condition, ORP values mostly present in the negative range. Some researcher reported that functioning anaerobic digester with healthy methane production seemed to thrive best at the ORP ranging between -520 and -530 mV (Fannin, 1987; Anderson et al., 2003).

2.1.2.8 Volatile fatty acid (VFA): VFA products in this case mean such as propionic, acetic and butyric acids. High accumulation of VFA is often associated with the effects of toxicity and some inhibition. In a healthy reactor, a typically VFA concentrations should be less than 100 mg/l as acetic (Anderson et al., 2003).

2.1.2.9 Sulfate-reducing bacteria (SRB): When the wastewater contains significant concentrations of sulfate, SRB will compete with methanogens for the same electron donors, acetate and H_2, and convert sulfate to sulfide. In this case, SRB could overcome the methanogens because of higher affinity; therefore, the COD/SO_4 ratios of higher 2.7 are recommended (Bitton, 2005).

2.1.4 Advantages and Disadvantages of the Anaerobic Process

The successful application of anaerobic processes both for digestion of sludge (biomass) and treatment of wastewater are recently notorious for quite a time. The rationale and interest in the use of anaerobic processes can be explained by considering the advantages and disadvantages of the process shown in Table 2.2.

Table 2.2 Advantages and disadvantages of anaerobic process.

Advantages	▪ Less energy required ▪ Less biological sludge production ▪ Fewer nutrients required ▪ Methane production, a potential energy source ▪ Smaller reactor volume required ▪ With acclimatization, most organic compounds could be transformed ▪ Rapid response to substrate addition after long period without feeding
Disadvantages	▪ Longer start-up time to develop necessary biomass inventory ▪ May require alkalinity and/or specific ion addition ▪ May require further treatment with an aerobic treatment process to meet discharge requirements ▪ May be more susceptible to upset due to toxic substances ▪ Potential for production of odors and corrosive gases

Source: Metcalf & Eddy (2004).

Although the anaerobic process have a number of advantages, the conventional anaerobic treatment, completely mix or plug flow digester, usually have several problems during operating and maintenance. One of major problems in conventional anaerobic system is sludge wash out; high volume of reactor is required in order to keep the biomass within the reactor as long as possible. Therefore, several modifications to solve this problem were attempted, such as returning the biomass into the reactor, or using a filter for trapping the biomass or even packing some media in the reactor. However, the cost of filter and packing material becomes the disadvantage. In addition, the problems of clogging, more pumping power required or the need of the packing level control and wasting with ingrowths were concerned. Subsequently, the UASB reactor was developed and seemed to be the right answer for the problems of sludge wash out and costly media. But the construction of efficient

gas-solid separate device and the need of reactor height are become bothersome and costly. Moreover, the complexity of sludge granulation is also a limitation to inexperienced or even experienced operators. Anaerobic baffled reactor (ABR), one type of anaerobic reactors developed from the rotating biological contactor (RBC) (Barber and Stuckey, 1999) may be able to resolve the above problems.

2.2 Anaerobic Baffled Reactor (ABR)

ABR, one type of high-rate anaerobic reactor initially developed since 1981 by McCarty and co-workers at Stanford University, USA (Barber and Stuckey, 1999). The ABR performance can be compared to a series of UASB (Up-flow Anaerobic Sludge Blanket), but does not require granulation of biomass and gas-solid separation device (Bachmann et al., 1985).

2.2.1 Design of the ABR

The reactor consists of a series of vertical baffles to force and direct the wastewater flow to downward and upward via the baffles from inlet through outlet. The biomass in the reactor rises and falls vertically within each compartment by gas production and flow of wastewater, but moves through outlet of the reactor with a slower horizontal velocity. Most of biomass will be retained in the reactor with the act of baffles. The original design and flow pattern of the first ABR as shown in Figure 2.3.

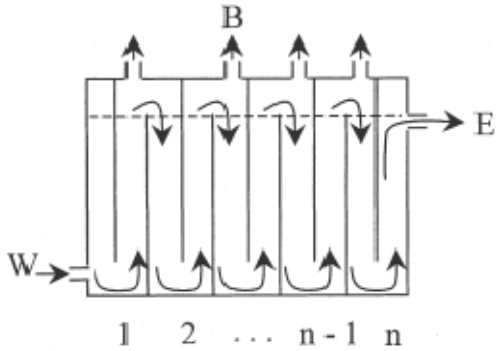


Figure 2.3 The original design of anaerobic baffled reactor (Barber and Stuckey, 1999).

ABR design is exceptional for the contact between substrate and bacteria without special mechanical mixing device. Mixing occurred in a reactor is caused by wastewater flow and biogas generation. In the case of low HRT, increasing of OLR, resulting in higher of gas production was reported to obtain a better mixing (Grobicki and Stuckey, 1992). The compartmentalized structure of ABR is a distinguished advantage that prevents sludge wash out; biomass will be retained in the reactor for a longer retention time. Consequently, SRT could be separated from HRT without needs of sludge granulation or filter or media packing. Accordingly, the reactor performance can be improved technically increase of SRT and economically by decrease of HRT (Nachaiyasit and Stuckey, 1997b; 1997c) and consequently reducing reactor volume.

In additional, the compartmentalized structure can act similar to a two-phase digestion process, which acidogenesis is separated from methanogenesis longitudinally. This will enhance stability and higher OLR of the anaerobic processes, also, increase the overall removal efficiency with shorter HRT (Demirer and Chen, 2005).

Despite the effectiveness of ABR mentioned above, in order to apply with various types of wastewater, several authors (Boopathy and Sievers, 1991; Skiadas and Lyberatos, 1998) have modified the reactor configurations to improve the performance. ABR modifications were summarized in Table 2.3.

Fannin et al. (1981) added vertical baffles to a plug flow reactor for enhancing solids retention, which allowed better substrate accessibility to methanogens (Figure 2.3a). Bachmann et al.(1983) narrowed down-flow chambers in order to reduce liquid vertical velocity and enhance solid retention in up-flow regions. The authors also slanted the edges of baffles for 40-45degree to help routing flow towards center of up-flow regions, resulting in the thorough mixing and reducing dead space in each compartment (Figure 2.3b). Tilche and Yang (1987) modified the last compartment as a settling zone to enhance solids retention, some packing media were positioned at the top of each compartment to prevent solids wash out and for easiness and

controllability of gas measurement, which indirectly enhanced reactor stability (Figure 2.3c) (Barber and Stuckey, 1999). Boopathy and Sievers (1991) did enlarge the first compartment in order to obtain better treatability of high solids wastewater (Figure 2.3d). Skiadas and Lyberatos (1998) applied the ABR with circular manner in the annular region between two concentric cylinders, and then called it "periodic anaerobic baffled reactor (PABR)". The influent and effluent ports are not fixed, resulting in flexibility of feed distribution into any compartment, which helps the biomass can withstand the stress coursed by overloading (Figure 2.3e).

Table 2.3 The modifications of the ABR.

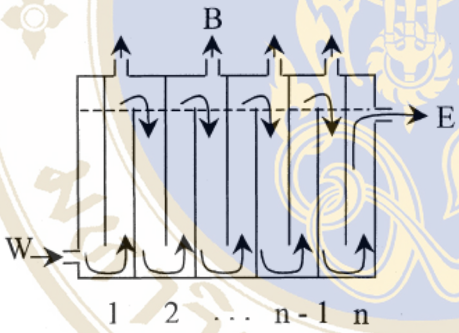
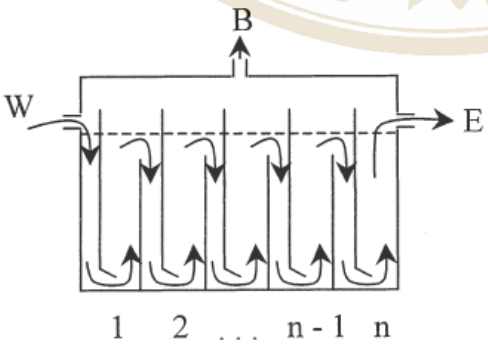
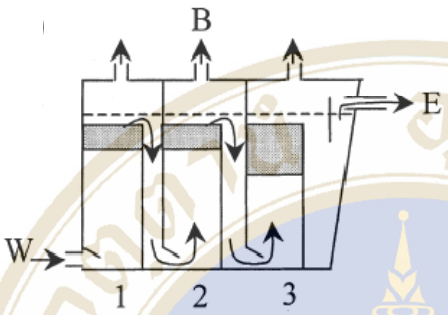
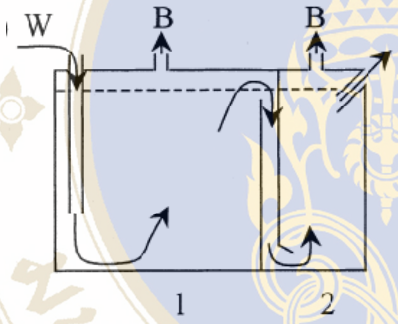
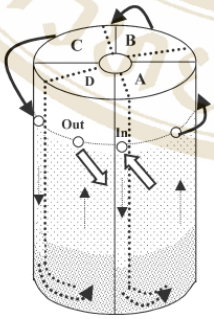
Figures	Modifications
(a) * 	Added vertical baffles to a plug flow.
(b) * 	Narrowed down-flow region and slanted edges on baffles (40-45°).

Table 2.3 The modifications of the ABR (continued).

Figures	Modifications
(c) * 	Settling the last compartment.
(d) * 	Enlargement of first compartment.
(e)** 	Apply the ABR into circular container as call “PABR”.

Remark: W= Wastewater inlet, B= Biogas, E= Effluent.

Sources: * Barber and Stuckey (1999).

** Skiadas and Lyberatos (1998).

As biomass retention can be accomplished when the up-flow velocity in each compartment is lower than the settling velocity, the position of baffles in each compartment obviously affect the retain ability of biomass within a reactor. The

closer to inlet of the baffle is, the slower up-flow velocity is. However, the problem of dead space may occur if the up-flow region is too large. Dama et al. (2001) recommended that the ratio of widths of down-flow to up-flow areas should be 1:3 to achieve in lowering up-flow velocities without increase of too much dead space.

Dead space is an extremely concern in the design of any wastewater treatment reactors including ABR. Several modifications to reduce the dead space have been attempted. A comparison of a straight-ended baffle with a slanted baffle was concluded by Dama et al. (2001) that the slanted baffle proven to be a better option to reduce the dead space in the up-flow region, and direct the flow through the central of compartment.

2.2.2 Advantages and Disadvantages of the ABR

Several advantages of ABR over other high-rate anaerobic reactors were recognized for quite a time. One of the significant advantages is its ability to separate acidogenesis and methanogenesis longitudinally, by itself, allowing the reactor to behave as a two-phase system without associated control problems and high costs.

Furthermore, the reactor design is comparatively simple, especially, there is no moving parts, gas-solid separated device or mechanical mixing required. Also, the complicated operations for biomass settling or sludge granulation are not necessary. Because of low sludge generation has been acknowledged as a common characteristic of anaerobic processes, high operating SRT is utterly necessary for efficient digestion and process stability. The ABR configuration can increase operating SRT without need of packing media, filter or any biomass trapping devices. In addition, gas separation is also not required, making its construction and operation are relatively inexpensive and simple compared with other types of anaerobic processes.

Since the SRT and HRT can be separately operated with the ABR configuration, unlike a continuously stirred tank reactor (CSTR) where its HRT and SRT are unavoidably equal, increase of wastewater treatment capacity and efficiency

can be possibly accomplished. Intermittent operation is also achievable, which would facilitate treatment of seasonal wastewaters. Moreover, the ABR configuration has been found to have higher resistance to hydraulic and organic shock loads, and also some toxic compounds contained in the influent.

The modest disadvantages of the ABR are similar to the other anaerobic designs. Pathogens and nutrients are only partially removed, consequently a post-treatment is required.

2.3 Fluorescence *in situ* Hybridization (FISH)

Nowadays, a fashionable method for microscopic identification is known as FISH technique, which was first developed in the late 1980s, however application in environmental sample was succeeded in the past decade ago. FISH using rRNA-targeted oligonucleotide probe is not only allows identification and enumeration on a culturaled microorganism, but can also reveal the complex microorganisms in their natural environment (Moter and Göble, 2000; Wagner et al., 2006) which was detected nucleic acid sequences by a fluorescently labeled probe that hybridizes specifically to its complementary target sequence within the intact cell. The procedure was shown in Appendix A.

2.3.1 FISH Application

In recently, FISH can useful be applied to wildly field such as, to analyze bacterial community structure and allows the direct identification and quantification of specific and/or general taxonomic groups of microorganisms within their natural microhabitat environmental sample such in sediments, seawater, root surfaces, drinking water, etc (Llobet-Brossa et al., 1998; MacNaughton et al., 1996; Alfreider et al., 1996; Kalmbach et al., 1999). Application of FISH in a wastewater treatment is usually used to identify group of microorganism in sludge to improve a system

performance (Schramm et al., 1999; Plumb et al., 2000; Kim et al., 2004; Sekiguchi et al., 1999).

2.3.2 Limitation of FISH

- Low signal intensity: Insufficiently probe penetrated into the bacterial cell consequent false negative results.
- Autofluorescence: Its can also found in material surrounding the bacteria in environment samples.
- Probe specific: Designate a specific oligonucleotide probe results in precision and reliability of FISH.
- Manual counting of fluorescent cells is often applied but fails for many organisms in sludge because cells in filaments or dense agglomerates cannot be distinguished.

2.3.3 Advantages of FISH

- Rapid
- Possible with an epifluorescence microscope which no need of an expensive confocal-laser-scanning microscope.
- Suitable for difficult matrix containing debris and cells of different fluorescence intensity.
- Suitable for FISH protocols requiring cell wall permeabilization.
- Allowing quantitative comparison between different concentrations of one organism as well as between different target organisms.
- Small uncertainty due to subjectivity of operators.
- Accurate enough to identify typical seasonal changes in the population composition.

2.4 Related Researches

In 1998, Barber and Stuckey investigated the starting up procedures of ABR, compared between gradually decrease of HRT and increase of substrate concentration. For the case of decrease of HRT, the authors initially operated the system with a long HRT (80 hrs) and designated substrate concentration (4,000 mg COD /l), then, gradually lowering the HRT to the target one (20 hrs) while keeping the substrate concentration constant. Another case is the system was initially operated with a short target HRT (20 hrs) and a low substrate concentration (1,000 mg COD /l), then, increase substrate concentration step-by-step to the designated one (4,000 mg COD/l). The authors concluded that the first procedure provided a greater stability than the latter, based on the improvement of solids accumulation, promotion of methanogenic populations and a faster recovery from hydraulic shocks.

Although the advantage of ABR over UASB is no needs of sludge granulation, a study by Freese and Stuckey (2000) showed that seed inoculation with granular sludge could reduce the overall start-up period for ABR. In comparison with using digested sludge as inoculated seed, the increase of OLR and decreasing of operating HRT were much more rapid.

The granulation ability of ABR was firstly observed by Tilche and Yang (1987) (cited by Boophthy and Tilche, 1992), then, Boopathy and Tilche (1992) also observed the pelletization of the biomass in a hybrid ABR treating molasses wastewater. A long term stability and low biomass wash out were also occurring during the study. Since sludge granulation can be established in ABR and help improve the performance of the ABR, Uyanik et al. (2002) and She et al. (2006) attempted to accelerate the granulation by adding polymer and granular activated carbon (GAC), respectively. The authors succeeded in granulating biomass, which consequently enhanced the OLR of the system.

Generally, the biological processes are affected by temperature, the optimum range is 25-35°C, but most of full-scale plants can not control operating temperature to the required one, therefore, numerous researchers studied the operating of ABR at low temperatures. Nachaiyasit and Stuckey (1997a) found that no significant reduction in overall COD removal efficiency when the temperature of the ABR was dropped from 35°C to 25°C, afterward, decrease of operating temperature to 15°C caused only 20% drop of COD removal efficiency. Also, Langenhoff and Stuckey (2000) operated the ABR with HRT of 10 hrs and feed concentration of 500 mg/l, also, gradually decreased the operating temperature from 35°C to 20°C and finally 10°C. The results shown that the COD removal efficiencies reduced from 95% to 70 % and 60 %, respectively. The author inferred that the ABR was possible for application both of low strength wastewater and low operating temperature.

ABR has been aware of its better ability to tolerate hydraulic and organic shocks. During a steady-state of the 8-compartment ABR operation with HRT of 20 hrs and OLR of 4.8 kg COD/m^3-d, Grobicki and Stuckey (1991) introduced a hydraulic shock load by decreasing the HRT to only 1 hr (consequently OLR became 96 kg COD/m^3-d) for a period of 3 hrs. The dropped COD removal was recovered to be over 96% within 24 hrs of resuming normal operating conditions while less than 15% of the active biomass was lost. Nachaiyasit and Stuckey (1997b; 1997c) reported that ABR endured organic shock better than hydraulic one. They studied with using 8-compartments ABR and launching the organic shock by increase the COD concentration implied as OLR from 4.8 to 18 kg COD/m^3-d within 20 days, this resulted in slightly decrease of COD removal efficiency from 98 to 90%. The study of hydraulic shock was conducted by lowering the HRT from 20 to 5 hr for 3 weeks, the baseline operating OLR of 4.8 kg COD/m^3-d increased to 19.2 kg COD/m^3-d, resulting in removal efficiency dropped to 52%. However, the system recuperated back to its baseline performance within 9 hrs after shock ceased.

The applications of ABR have been studied by several authors; Boopathy et al. (1988) and Akunna and Clark (2000) performed an ABR treating the concentrated

distillery wastewater contained COD concentration of 51,000 mg/l and 9,500 mg/l from a Scotch whisky factory respectively. They achieved the removal efficiencies up to 90%. Polprasert et al. (1992) investigated by using 4-compartments ABR to treat a slaughterhouse wastewater contained COD concentrations of 480-730 mg/l, and reported COD removals were up to 75% with operating HRT of 26 hrs.

Boopathy and Sievers (1991) modified ABR to treat high strength swine waste contained 51.7 g/l total solids. The removal of COD reached to 80% under operating HRT of 15 days. Based on this study, the authors found that the operating SRT to be higher than 20 days was possible. Boopathy (1998) studied the overall performance of ABR treating whole swine waste and reported that the COD reduction was in the range of 70-78%. Moreover, they concluded that the ABRs with two, three, four and five compartments accomplished the SRTs of 25, 30, 36 and 42 days, respectively, under operating HRT of 14 days.

The performance of ABR treating molasses wastewater was investigated by several researchers (Boopathy and Tilche, 1991; Boopathy and Tilche, 1992; Vossoughi et al., 2003). These investigations mostly achieved 45-98% COD removal efficiencies with various operating conditions (Table 2.4). Besides, the ABR was also efficient for decolorization of dye contaminated wastewater, especially, Bell et al. (2000) illustrated that their ABR could perform the 95% color reduction. The other applications of ABR in recent studies are shown in Table 2.4.

The lack of ability in nutrient removal is a weakness of common anaerobic processes. However, Barber and Stuckey (2000a; 2000b) deleted this limitation by modification the ABR to serve nitrification-denitrification process. This modification was addition of aeration in penultimate compartment to permit the nitrification, oxidation of ammonia to nitrate, and recycle the nitrate back into the inlet for conversion of nitrate to nitrogen gas via denitrification. They achieved the nitrogen removal as high as 96% since the second compartment.

Herein these reviews, pilot-scale applications of ABR were only performed with domestic wastewater treatment. The 3,200 liters of ABR was implemented for treatment of domestic wastewater in South Africa, which its influent contained with COD ranging between 350 and 1,200 mg/l. With the operating HRT of 20 hrs, 70-80% COD reduction efficiencies were achieved (Dama et al., 2002). In addition, Foxon et al. (2004) conducted the 3,000 liters ABR treating domestic wastewater and obtained the removal efficiency up to 72% with 22 hrs HRT.

Nowadays, the full-scale ABR was also applied for treating domestic wastewater at Tenjo, Columbia (Barber and Stuckey, 1999). However, the ABR is shown the appropriation for treating industrial wastewater as it can withstand severe hydraulic and organic shocks, intermittent feeding and temperature changes. Furthermore, its inherent two-phase behavior provides greater resistance to toxicity in comparison with other well-established technologies.

Table 2.4 Performance data on anaerobic baffled reactor[a] reviewed by Barber and Stuckey (1999).

Substrate	Vol. (L)	Compartment	Biomass (g VSS/L)	Inlet COD (mg/l)	Loading rate (kg/m^3–d)	COD removal (%)	HRT (hrs)	Temp.	References
Undiluted sea kelp	9.8	5		6,000-36,000	0.4-2,4		360	35	Chynoweth et al., 1980
Dilute sea kelp	10	4			1.6			35	Fannin et al., 1981, 1982
	10	4		67,200-89,600	5.6-6.4		288-366	35	
	10	4		80000	1.6		1200	35	
Carbohydrate-protein	6.3	5		7,100-7,600	43862	79-82		35	Bachmann et al., 1983
Carbohydrate-protein	6.3	5		8000	2.5-3.6	55-93	4.8-71	35	Bachmann et al., 1985
Carbohydrate-protein	10	8		4000	1.2-4.8[b]	99	20	35	Grobicki and Stuckey, 1989
Carbohydrate-protein	7.8-10.4	4-8		4000	1.2-4.8	95	20-80	35	Grobicki and Stuckey, 1991
Carbohydrate-protein	10	4-8	0-8.5	4000			29221	35	Grobicki and Stuckey, 1992
Carbohydrate-protein	10	8	18	4000	1.2-4.8	98,93	20,80	35	Nachaiyasit and Stuckey, 1996
Carbohydrate-protein	10	8	18	4000	1.2-4.8	75-83, 93-97, 96	20, 20, 20	15, 25, 35	Nachaiyasit and Stuckey, 1997a
Carbohydrate-protein	10	8	18	4000	4.8-9.6	90-98	20	35	Nachaiyasit and Stuckey, 1997b
Carbohydrate-protein	10	8	18	4000	4.8-18	52-98	18264	35	Nachaiyasit and Stuckey, 1997c
Carbohydrate-protein	10	8	18	1,000-4,000	1.2-4.8	98	20-80	35	Barber and Stuckey, 1998
Sucrose	75	11		344-500	0.7-2	85-93	39422	13-16	Orozeo, 1998
Sucrose*	90	4	≈41	1,500-3,000	1.2-4.5	87-92	20	34	She et al., 2006

Table 2.4 Performance data on anaerobic baffled reactor[a] reviewed by Barber and Stuckey (1999) (continued).

Substrate	Vol. (L)	Compartment	Biomass (g VSS/L)	Inlet COD (mg/l)	Loading rate (kg/m^3-d)	COD removal (%)	HRT (hrs)	Temp.	References
Glucose	6	5		1,000-10,000	2-20	72-99	12	35	Bae et al., 1997
Pharmaceutical wastewater	10	5		20,000	20	36-68	24	35	Fox and Venkatasubbiah, 1996
Phenolic		5	20-25	2,200-3,192	1.67-2.5	83-94	~24	21	Holt et al., 1997
Synthetic greywater	8	6		480	0.1-0.4	63-81	48-84	25-33	Witthauer and Stuckey, 1982
Wisky distillery wastewater	6.3	5		51,000	2.2-3.5	90	360	30	Boopathy, 1988
Wisky distillery wastewater*	35	10		9,500	0.99, 1.33, 2.37, 4.75	93-96	10, 7, 4, 2	37	Akunna and Clark, 2000
Brewery wastewater*	10	5		115,000-125,000	2.16-13.38	93-96	55.5	35	Baloch et al., 2007
Slaughterhouse wastewater	5.16	4		450-550	0.9-4.3	75-90	2.5-26	25-30	Polprasert et al., 1992
Municipal wastewater	350	3		264-906	2.17	90	4.8-15	18-28	Garuti et al., 1992
Domestic sewage industrial waste	394,000	8		315c	0.85	70	10.3	15	Orozco, 1997
Domestic wastewater*	3,200	8		350-1,200		70-90	20		Dama et al., 2002
Domestic wastewater*	3,000	8		<200		58-72	22		Foxon et al., 2004
Molasses wastewater	150	3	5.3	5,000-10,000	5.5	98		37	Yang et al.,1988
Molasses wastewater	150	3	4.01	115,771-990,000	4.3-2.8	49-88	138-850	37	Boopathy and Tilche, 1991
Molasses wastewater	150	3	4.01	115,771-990,000	20	70	~138	37	Boopathy and Tilche, 1992

Table 2.4 Performance data on anaerobic baffled reactor[a] reviewed by Barber and Stuckey (1999) (continued).

Substrate	Vol. (L)	Compartment	Biomass (g VSS/L)	Inlet COD (mg/l)	Loading rate (kg/m^3-d)	COD removal (%)	HRT (hrs)	Temp.	References
Molasses wastewater	150	3		115,771-990,000	10	40-75	24-144	37	Xing and Tilche, 1992
Molasses wastewater	150	3	4.11, 7.21	115,771-990,000	20	>70	~140	37	Xing et al.,1991
Molasses-sulfate (synthetic) *	10	5		1,800, 3,000		86	24	35	Vossoughi et al., 2003
Dilute swine wastewater	20			<5,000	1.8	75	60	30	Yang and Moengangongo, 1987
Swine manure	15			58,000	4	62-69	360	35	Boopathy and Sievers, 1991
Whole swine waste*	15	8		59,400		70-78	14	35	Boopathy, 1998
Dye wastewater*	10	8	12	1,000	1.2	50-60, ~70	20	35	Bell et al., 2000
Dye wastewater*	10	8	12	1,000	1.2	70-80[d]	20	35	Plumb et al., 2001
Dye wastewater*	10	8	20	4,000	4.8	>90	20	35	Bell and Buckley, 2003
Dyeing wastewater*	10	5		1,201.7		86.6	12	17.8	Wu et al., 2007
Soluble and colloidal wastewater*	10	8	6.5-19	500		60->95	10	35, 20, 10	Langenhoff and Stuckey, 2000
Soluble and colloidal wastewater*	10	4		500		40->95	1.3-80	35	Langenhoff et al., 2000
Ice cream wastewater*	120	3		18,000-21,000	0.9-10.5	>95	2		Uyahik et al., 2002
Palp and paper mill wastewater*	10		22	4,000	5	60	48	35	Grover et al., 1999

Remark:
[a] Contains calculated results, either from graphs or from supplied data.
[b] Also with shock loading of 96 kg/m^3-d.
[c] BOD_5 value.
[d] TOC value.
* More revision by this study.

CHAPTER III
MATERIAL AND METHODS

This study was to investigate the effect of compartment numbers on SRT and SRT/HRT ratio, and determine the organic loading rate (OLR) of the anaerobic baffled reactor (ABR) for treating carbohydrate-protein wastewater. Reactors used in this study were three difference numbers of compartment, i.e. 3-compartment (3C), 6-compartment (6C), and 8-compartment (8C). By varying the three compartment numbers and four OLRs, six experiments were set up for this study. All of the experiments were conducted and investigated at a laboratory of the Department of Sanitary Engineering, Faculty of Public Health, Mahidol University.

3.1 Wastewater Preparation

Wastewater used in this study was mainly synthesized with carbohydrate and protein substances, sucrose and nutrient broth (HIMEDIA®), respectively. This synthetic wastewater was daily prepared with tap water and some other nutrients (as shown in Table 3.1). Wastewater contained constant COD concentration of 4,000 mg/l for the experiments to investigate the effect of compartment numbers. For the experiments to determine an optimum OLRs, COD concentration in the feed was varied of 8,000, 12,000, and 16,000 mg/l.

3.2 Experimental Setup

A schematic diagram of experimental setup was shown in Figure 3.1. The system consists of ABRs, peristaltic pumps, storage containers, effluent containers, and gas meters.

Table 3.1 Compositions of synthetic carbohydrate-protein wastewater (4,000 mg/l).

Component	Concentrations
Sucrose	3,000 mg/l
Nutrient broth	1,500 mg/l
$NaHCO_3$	3,000 mg/l
$CaCl_2$	100 mg/l as Ca
KCl	200 mg/l as K
$MgSO_4 \cdot 7H_2O$	75 mg/l as Mg

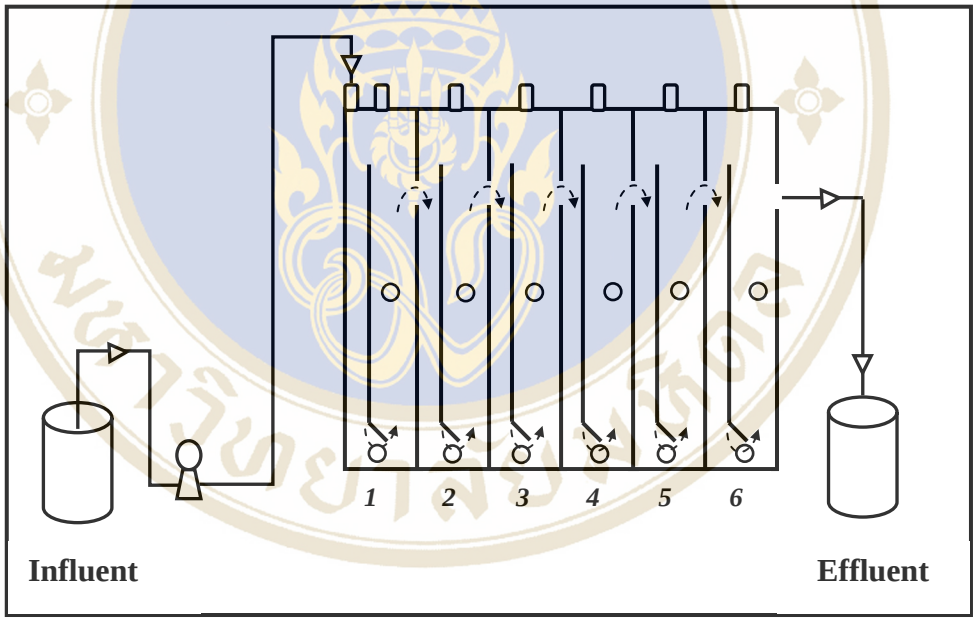


Figure 3.1 A schematic diagram of experimental setup (6-compartments).

- **Reactors:** Three laboratory-scale ABRs were made of clear acrylic with the detail and dimension of reactors as shown in Figure 3.2. All three reactors were having ten liters effective volume and most of the components are similar. The difference was the number of compartment consisted in each reactor, which 3, 6, and 8 compartments were applied for this study. Each compartment has a vertical baffle that directs the liquid flow alternately downward and upward. The position of baffle

that is closer to the inlet is result in lower up-flow velocity and consequently reduces solids carryover. Therefore, the ratio of down-flow and up-flow width in each compartment used in this study is 1:3 similar to Dama et al. (2002) suggested. Also, the 45-degree slanting baffle was recommended to reduce the region of dead space and direct the flow to the center of the up-flow region (Dama et al., 2002). The wastewater flows from one compartment to the next through window cut on the acrylic partition. The gas outlets are on the upper part of the reactor and sampling ports are at the side.

- **Peristaltic pump:** Peristaltic pump was used to feed wastewater into the reactor.

- **Gas meter:** Gas meter used in this study based on liquid displacement method.

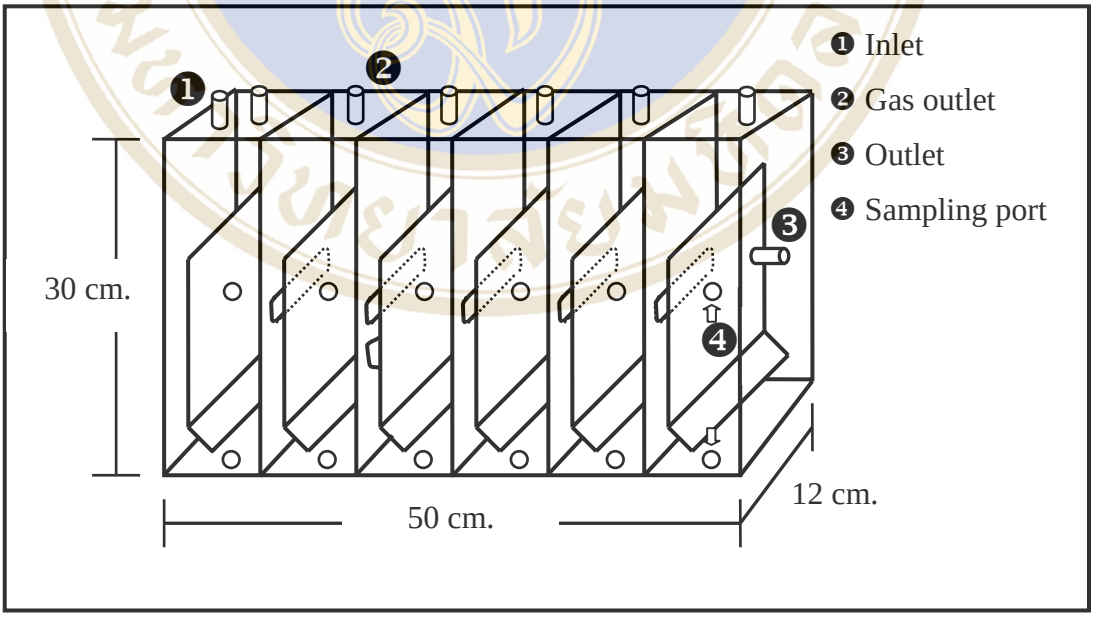


Figure 3.2 Details and dimension of experimental reactor (6-compartments).

3.3 Experimental Procedure and Design

Experimental procedures were composed of seeding-acclimatization phase and designated experiments phase in detail as shown in Figure 3.3.

3.3.1 Seeding and Acclimatization

The systems were inoculated with anaerobic sludge from ABR treating swine wastewater with COD concentration of 4,000 mg/l for approximately 3 months. Seed sludge (10 liters) was fed into each reactor, which total MLSS concentration about of 28 mg/l and then left sludge settled for about two days. For acclimatization step, the system was fed with synthetic carbohydrate-protein wastewater contained with the designed feed strength in each experiment and operated at initial HRT of 80 hrs, which is suggested to result in high stability and COD removal (Barber and Stuckey, 1998), and then the HRT was gradual decrease to the designated HRT of 24 hrs. The system was operated until a steady state, which defined as the occurring when the variations of the COD removal efficiency and the effluent solid concentration are less than 5%, is achieved continuously at least for one week.

3.3.2 Experimental Design

Experiment was divided into two parts, in the first part, three reactors name as, 3C-OLR4, 6C-OLR4, and 8C-OLR4 were operated of 4 g COD/l-d OLR and HRT of 24 hrs, but the numbers of compartment were difference of 3, 6, and 8 compartments, respectively. The second part was select the reactor with eight-compartment reactor in consideration of resulted from the first part and operated under different three OLRs i.e, 8, 12, and 16 g COD/l-d (or name as OLR8, OLR12, and OLR16, respectively). Therefore, six experiments were conducted with operating conditions as shown in Table 3.2.

Table 3.2 The operation conditions in this study.

Experimental names	Number of compartments	COD (mg/l)	HRT (hrs)	OLR (g COD/l-d)
Part I: Effect of compartment numbers on SRT and SRT/HRT ratio.				
3C-OLR4	3	4,000	24	4
6C-OLR4	6	4,000	24	4
8C-OLR4	8	4,000	24	4
Part II: Optimum organic loading rate.				
8C-OLR4	8	4,000	24	4
8C-OLR8	8	8,000	24	8
8C-OLR12	8	12,000	24	12
8C-OLR16	8	16,000	24	16

3.4 Analytical Methods

Samples were regularly collected by grab sampling method. Influent and effluent sample were collected from storage container. Supernatant of each compartment was collected from sampling ports as shown in Figure 3.2. The parameters, tCOD, sCOD, Alkalinity, TSS, VSS, VFA, pH, and ORP was performed according to Standard Methods (APHA et al., 1998) as listed in Table 3.3, and sampled depend on parameters (Table 3.4). Biogas was collected and counted by gas meters using water displacement method. SRT was calculated using equation 1.1. Bacterial cell was determined by FISH technique (procedure shown in Appendix A).

$$\text{SRT (d)} = \frac{\text{Mass in reactor (g)}}{\text{Sludge washout rate (g/d)}} \qquad (1.1)$$

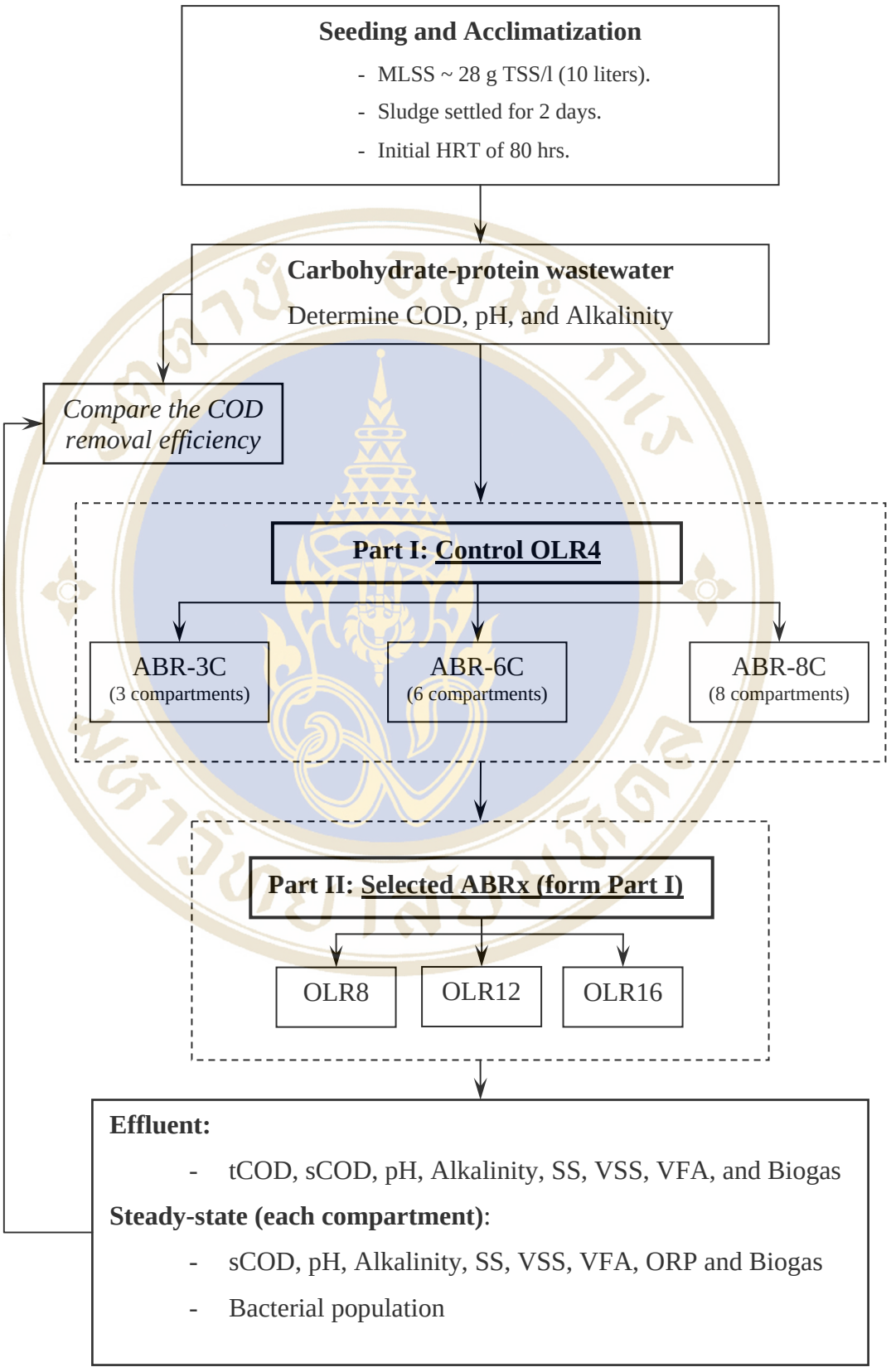


Figure 3.3 Experimental procedures.

Table 3.3 Analytical methods.

Parameters	Methods	References
pH	Electrometric Method	APHA et al., 1998
Alkalinity	Titration Method	APHA et al., 1998
COD	Closed reflux, Titrimethod	APHA et al., 1998
SS	Total suspended solid dried at 103-105°C	APHA et al., 1998
VSS	Volatile suspended solid ignited at 550°C	APHA et al., 1998
VFA	Titration Method	APHA et al., 1998
ORP	Electrode Method	APHA et al., 1998
Biogas	Water displacement method	
Bacterial cell	Fluorescence in situ hybridization	Jupraputtasri et al., 2005

Table 3.4 The measured parameters, sampling points.

Parameters	Unit	Influent	Effluent	Compartment
pH		✓	✓	✓
tCOD	mg/l	✓	✓	×
sCOD	mg/l	×	✓	✓
TSS	mg/l	✓	✓	✓
VSS	mg/l	✓	✓	✓
Alkalinity	mg/l as $CaCO_3$	✓	✓	✓
VFA	mg/l as $CaCO_3$	×	✓	✓
ORP	mV	×	×	✓
Biogas	liter	×	×	✓
Bacteria	-	×	×	✓

Remark : ✓ Measure

× Not measure

CHAPTER IV
RESULTS AND DISCUSSION

This experiment was to investigate optimum SRT/HRT ratio and organic loading rate (OLR) of anaerobic baffled reactor (ABR) treating carbohydrate-protein wastewater. The study was divided into two parts; *Part I* was investigated the effect of compartment numbers on SRT and SRT/HRT ratio which consisted of three experimental setups with different compartment numbers i.e. 3, 6, and 8 compartments. This part was operated to the purpose HRT of 24 hrs with OLR of 4 g COD/l-d (OLR4). Then the reactor with appropriate numbers of compartment in consideration of results in this part was selected to further investigate the optimum OLR in the next part. *Part II* was operated with same HRT as in the first part, but varied three different OLRs, i.e. 8, 12, and 16 g COD/l-d (or named OLR8, OLR12, and OLR16, respectively). All experiments were operated and conducted at room temperature within the laboratory of the Department of Sanitary Engineering, Faculty of Public Health, Mahidol University.

Some abbreviation were used in this chapter occasionally, i.e. an acronym C was an abbreviation of the word “Compartment” and the number after represented the “sequence of compartment” in ABRs, such as, C1 refer to the first compartment, C2 refer to the second compartment, C4 refer to the fourth compartment, and so on. Furthermore, in order to easily understand the condition of each experiment, some abbreviations were used to represent the ABRs, referring to their operating conditions (Table 4.1).

Table 4.1 The details of experiments.

Experimental names	Numbers of compartment	OLR (g COD/l-d)
Part I : Effect of compartment numbers on SRT and SRT/HRT ratio.		
3C-OLR4	3	4
6C-OLR4	6	4
8C-OLR4	8	4
Part II: Optimum organic loading rate.		
8C-OLR4	8	4
8C-OLR8	8	8
8C-OLR12	8	12
8C-OLR16	8	16

4.1 Seeding and Acclimatization

Acclimatization of sludge is a very important step in biological wastewater treatment, especially in anaerobic processes. Good start-up prepares microorganism structure proper for wastewater source, resulting in more reactor capability and performance. In addition, adequate seeding is a vital importance due to the slow growth of anaerobic microorganisms, resulted in reducing a start-up period.

For this study, the reactors were initially inoculated with anaerobic sludge from the ABR process treating swine wastewater with COD concentration of 4,000 mg/l for approximately 3 months. As a purpose to inoculate seed sludge of about fifty percent of effective volume, ten liters of seed sludge with MLSS concentration of around 28 g TSS/l (Nachaiyasit and Stuckey, 1996; 1997a; 1997b, 1997c; Barber and Stuckey, 1998) was fed to the reactor and left sludge settled for about two days. Then, synthetic carbohydrate-protein wastewater was fed with initial operating HRT of 80 hrs till pseudo-steady-state was reached. The HRT decreased to 48 hrs till the system was monitored as pseudo-steady-state again, and then adjusted to the designed HRT of 24 hrs. After operating with 24 hrs HRT, the steady-state was expected in consideration of constant COD remove efficiency and effluent TSS. As mention

above, seed sludge was acclimatized by gradually increase OLR, that resulted in promoting development of the slowly anaerobic microorganism population (Chynoweth and Isaacson, 1987; Henze and Harremoes, 1987; Angelidaki et al., 2006). In this study increasing of OLRs was done by decreasing HRT, which was reported to obtain faster recovery than an increasing feed concentration (Barber and Stuckey, 1998). One of the main reasons is the initial operating with high HRT helps reduce sludge washout during acclimatization period.

Table 4.2 Initial seed sludge concentration.

Experiments	TSS (mg/l)	VSS (mg/l)	VSS/TSS
3C-OLR4	28,800	19,600	0.7
6C-OLR4	27,300	21,100	0.8
8C-OLR4	27,500	21,500	0.8
8C-OLR8	27,700	23,100	0.8
8C-OLR12	28,700	20,100	0.7
8C-OLR16	27,600	21,200	0.8

Table 4.2 shows the concentration and volatile fraction quality of seed sludge in each experiment. Initial seed sludge concentration of 28,000 mg/l (20,000 mg VSS/l) was controlled to the same in all experiments. The VSS/TSS ratio were between 0.7-0.8 which indicated that the sludge possibly has a high active mass (organic>inert).

4.2 Part I: Effects of Compartment Numbers on SRT and SRT/HRT Ratio

An anaerobic microorganism has tended to slowly growth, consequently maintaining biomass in a reactor is a significant part of the anaerobic operation. ABR is one of high rate anaerobic processes that can retain higher biomass by its compartmentalized structure, hence, the effect of compartment numbers on SRT and SRT/HRT ratio was considered.

Table 4.3 Performance of the ABRs during steady-state in Part I.

Parameters		3C-OLR4		6C-OLR4		8C-OLR4	
		Inf [c]	*Eff* [d]	*Inf*	*Eff*	*Inf*	*Eff*
pH	Mean	8.2	7.9	8.1	7.9	8.1	7.9
	SD.	*0.04*	*0.21*	*0.25*	*0.10*	*0.25*	*0.20*
tCOD[a]	Mean	4,050	1,248	4,230	1,377	4,230	1335
(mg/l)	*SD.*	*166.9*	*79.4*	*137.1*	*37.5*	*126.5*	*59.0*
sCOD[b]	Mean		1,066		929		731
(mg/l)	*SD.*		*29.5*		*18.8*		*17.2*
COD removal	Mean		74		78		83
efficiency *(%)*[e]	*SD.*		*0.9*		*0.4*		*0.8*
COD utilization	Mean		29.8		32.8		35.0
rate *(g/d)*	*SD.*		*1.5*		*1.2*		*1.3*
TSS	Mean		284		218.1		203.4
(mg/l)	*SD.*		*60.0*		*66.9*		*46.1*
VSS	Mean		211		177.1		148.8
(mg/l)	*SD.*		*38.8*		*66.6*		*40.0*
Slugde washout	Mean		2.8		2.1		2.0
rate *(g TSS/d)*	*SD.*		*6.0*		*6.6*		*4.6*
Alkalinity	Mean	2,220	2,206	2,040	1,948	2,040	2,048
(mg/l as $CaCO_3$)	*SD.*	*51.2*	*161.3*	*104.9*	*61.1*	*104.9*	*61.1*
VFA	Mean		390		333		353
(mg/l as $CaCO_3$)	*SD.*		*55.8*		*92.6*		*96.1*
VFA/Alkalinity	Mean		0.2		0.17		0.2
ratio	*SD.*		*0.02*		*0.04*		*0.05*
Biogas volume			10		12		13
(l/d)			*1.1*		*1.1*		*1.1*

Note: [a] total COD [b] soluble COD

[c] Influent [d] Effluent

[e] $\frac{(\text{tCOD}_{\text{Inf}} - \text{sCOD}_{\text{Eff}}) \times 100}{\text{tCOD}_{\text{Inf}}}$

4.2.1 Wastewater Characteristic

This section shows the results and discussion of the overall performance since the acclimatization period to the steady-state of the experiments 3C-OLR4, 6C-OLR4, and 8C-OLR4. The influent and effluent characteristics were summarized in Table 4.3.

Synthetic carbohydrate-protein wastewater used in this part was daily prepared as the composition shown in Table 3.1.

An average influent COD of the 3C-OLR4, 6C-OLR4, and 8C-OLR4 experiments were 4,050, 4,230, and 4,230 mg/l, respectively. The pH values of the feeds were 8.2, 8.1, and 8.1 with prepared alkalinity of 2,220, 2,040, and 2,040 mg/l as $CaCO_3$, respectively (Table 4.3). Influent COD concentration all ABRs in this part were nearly 4,000 mg/l, while average pH was slightly above 8.0. This influent was prepared with average alkalinity was around 2,000 mg/l as $CaCO_3$, which was found to be adequate for maintaining the system performance.

4.2.2 The Overall COD Removal Efficiency

The COD removal efficiency of all three experiments were shown in Figure 4.1. The 3C-OLR4 experiment was initially operated at 80 hrs HRT with the fed wastewater contained COD of 4,000 mg/l (or OLR 1.2 g COD/l-d) for 20 days, the COD removal efficiency of about 85% was obtained during this period (Figure 4.1a). When HRT decreased to 48 hrs (or OLR 2 g COD/l-d) COD removal efficiency rapidly decreased during the first ten day after HRT adjusting, then, gradually maintained at the constant efficiency around 65%. The HRT was adjusted again to the designed one of 24 hrs (or 4 g COD/l-d). The 74% average COD removal efficiency could be achieved.

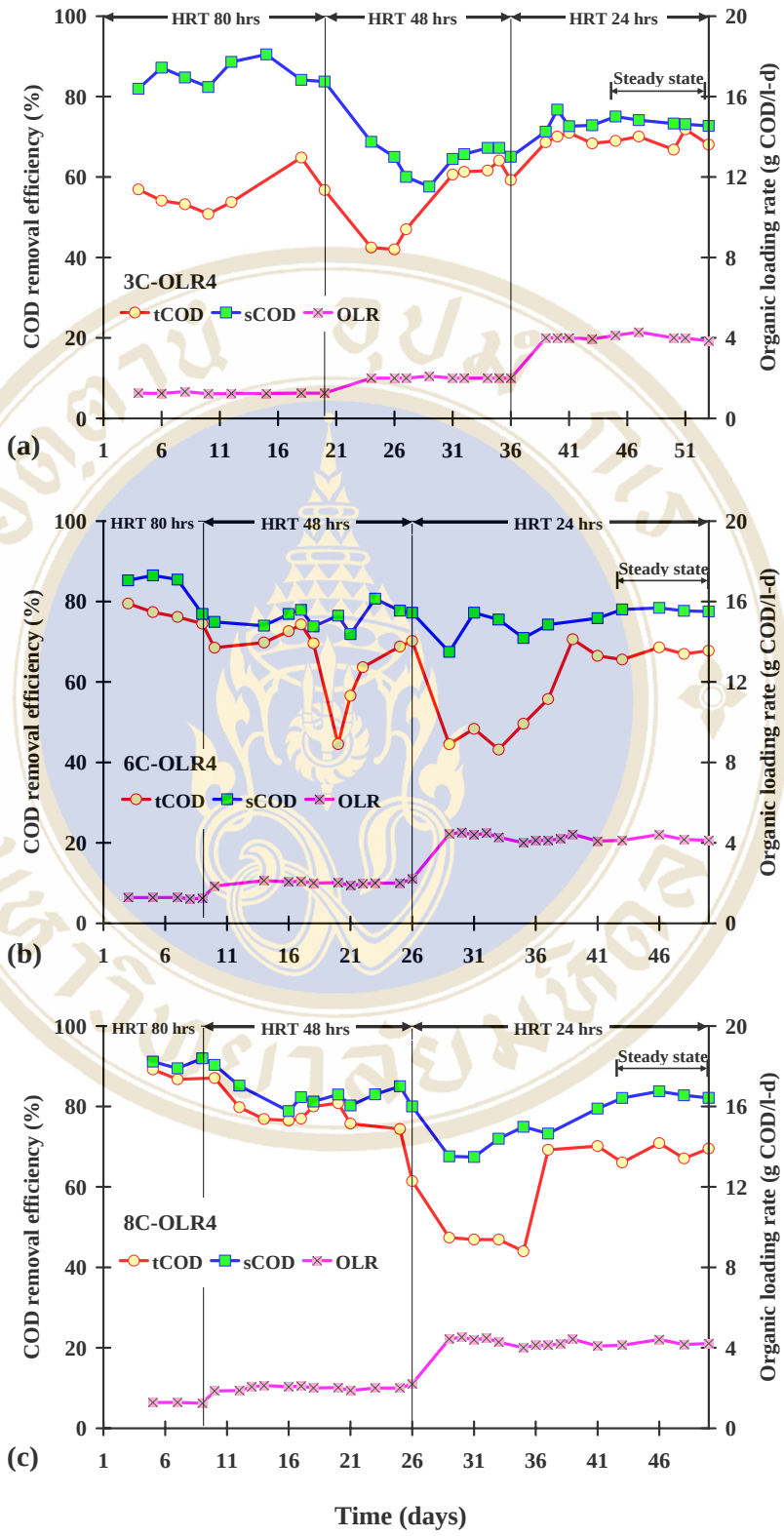


Figure 4.1 Overall COD removal efficiency and organic loading rate of the experiments; (a) 3C-OLR4 (b) 6C-OLR4 (c) 8C-OLR4.

Figure 4.1b shows the COD removal efficiency of the 6C-OLR4 experiment, which was operated with 80 hrs HRT and influent COD of 4,000 mg/l (or OLR 1.2 g COD/l-d) and received COD removal efficiency around 84%. When decreasing HRT to 48 hrs (or OLR 2 g COD/l-d), COD removal efficiency was obtained at about 76% at the steady state of 24 hrs HRT operation, COD removal efficiency could be maintained near 78%.

The 8C-OLR4 was operated with 80 hrs HRT (or OLR 1.2 g COD/l-d) for 9 days, COD removal efficiency in this period was averagely 91%. During day 10-26, HRT was decreased to 48 hrs (or OLR 2 g COD/l-d) and COD removal efficiency was maintained at 83%. After HRT was finally adjusted to 24 hrs, average COD removal efficiency at steady-state of 83% has achieved as presented in Figure 4.1c.

The investigation of the COD removal efficiencies of ABRs with various numbers of compartment found that the initial removal efficiency was quite high (>80% removed) in all ABRs at HRT of 80 hrs. The removal efficiency decreased when HRT increased because decreasing HRT (or increasing OLR) resulted in higher sludge washout. Moreover, lower HRT induced channeling occurrence, resulting in less contact between sludge and substrate (Grobicki and Stuckey, 1991). In addition, tCOD removal efficiency was related to total suspended solid (TSS) in the effluent, high effluent TSS affected in lowering tCOD removal efficiency (data about solid will be discussed in detail in Topic of “solid retention time”). COD utilization rate (g/d) was tabulated in Table 4.3, which average COD utilization rates of the 3C-OLR4, 6C-OLR4, and 8C-OLR4 experiment were 29.8, 32.8, and 35.0 g/d, respectively. This could be said that more compartments could enhance COD utilization rate. The performance of each compartment was summarized in Table 4.4.

Interestingly, the COD removal efficiency of 3C-OLR4 experiment was 20% reduced when adjusting HRT from 80 to 48 hrs (or increasing OLR from 1.2 to 2 g COD/l-d), while 6C-OLR4 and 8C-OLR4 experiments were only 10% reduced, also the same operating OLR (or the same HRT) the experiments with higher numbers of compartment was maintained higher COD removal efficiency (Figure 4.2). It could

be stated that the ABR with more numbers of compartment could be more tolerated to the hydraulic changing than lower numbers of compartment.

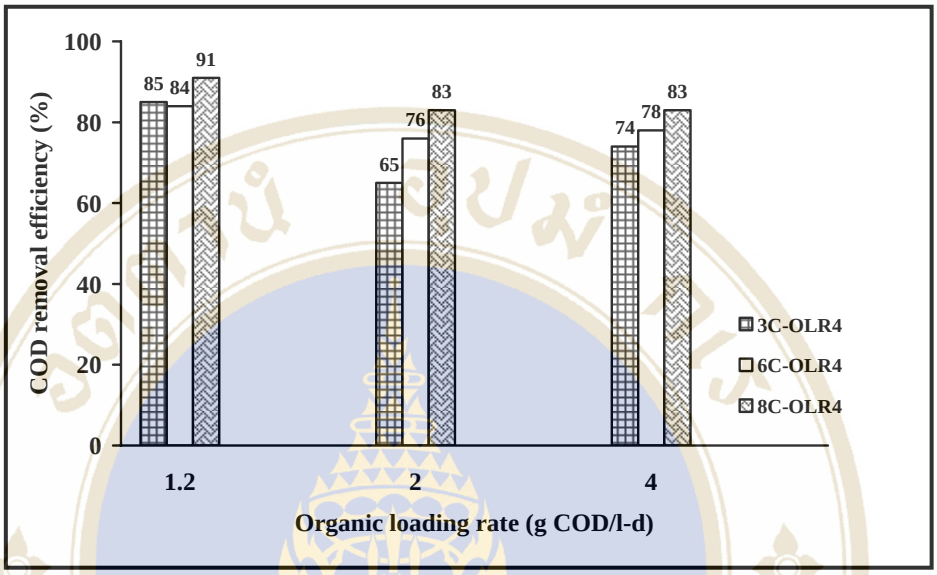


Figure 4.2 COD removal efficiencies of the Part I experiment with various OLRs.

Figure 4.3 shows the sCOD concentrations and the removal efficiencies in each compartment during the steady-state period. The sCOD concentration of 3C-OLR4 (Figure 4.3a) decreased from 4,000 mg/l in the influent to about 2,380 mg/l (~40% removed) in C1 (first compartment) and gradually decreased to an approximately of 1,030 mg/l (~74% removed) in C3 (third compartment). For the 6C-OLR4 experiment, the sCOD concentration decreased gradually from 1,730 mg/l in C1 (~58% removed) and to 970 mg/l at C6 (~78% removed). For the 8C-OLR4 experiment, sCOD was removed from the influent to about 1,900 mg/l in C1 (~56% removed) and to approximately 830 mg/l in C8 (~80% removed) as shown in Figure 4.3b and 4.3c, respectively.

The results from Table 4.4 and Figure 4.3 show that most of COD was removed in the first compartment while in the latter compartments, percentages of the COD removed slightly increased through lengthwise. The reason of this is the chemical structure of non-degradable organic matter in the latter compartment was shifted.

Table 4.4 Average profile performance at steady-state of ABRs in the Part I experiment.

	No.[a]	pH	sCOD (mg/l)	Efficiency (%)	Alkalinity (mg/l as $CaCO_3$)	VFA (mg/l as $CaCO_3$)	VFA/ Alk.	ORP (mV)	TSS (mg/l)
3C-OLR4	C1	6.8	2,379	40	1,972	922	0.5	-314	11,020
	C2	6.9	1,684	58	1,995	808	0.4	-324	9,092
	C3	7.1	1,032	74	2,067	677	0.3	-353	9,951
6C-OLR4	C1	6.8	1,730	58	1,823	567	0.3	-310	18,890
	C2	6.9	1,450	65	1,810	587	0.3	-324	15,747
	C3	6.9	1,330	68	1,853	503	0.3	-330	13,945
	C4	7.0	1,250	70	1,910	437	0.2	-331	13,840
	C5	7.3	1,130	73	2,018	395	0.2	-342	13,966
	C6	7.5	970	77	2,033	353	0.2	-349	15,770
8C-OLR4	C1	6.5	1,873	56	1,487	833	0.6	-390	27,867
	C2	6.7	1,637	61	1,620	835	0.5	-318	23,833
	C3	6.7	1,480	65	1,698	739	0.4	-332	23,867
	C4	6.8	1,410	67	1,713	690	0.4	-344	22,767
	C5	7.1	1,245	72	1,787	665	0.4	-355	21,233
	C6	7.2	1,005	76	1,788	622	0.3	-360	20,433
	C7	7.5	915	78	1,855	595	0.3	-352	17,167
	C8	7.7	834	80	1,893	562	0.3	-347	18,900

[a] = Compartment numbers.

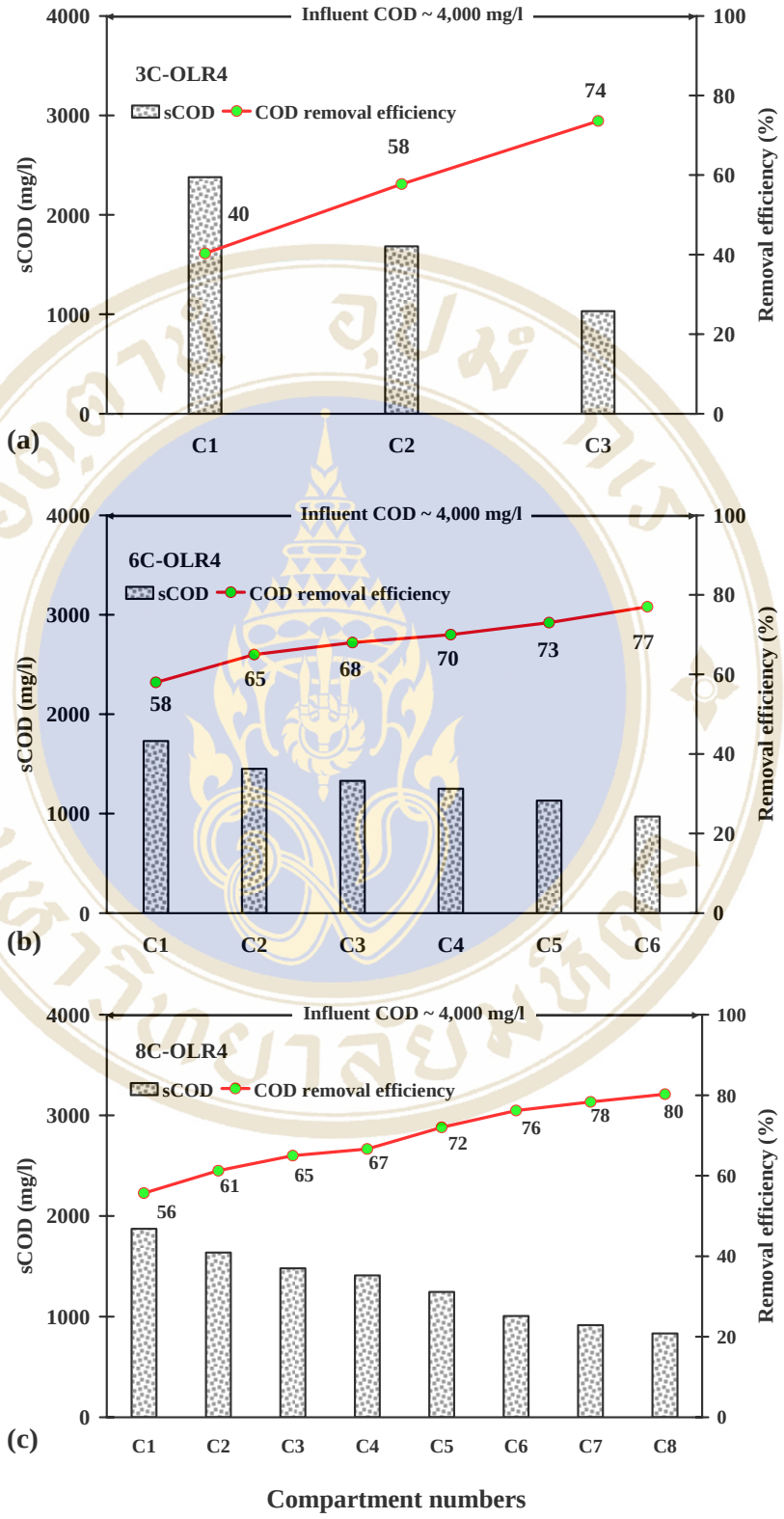


Figure 4.3 COD profiles in each compartment during steady-state of the experiments; (a) 3C-OLR4 (b) 6C-OLR4 (c) 8C-OLR4.

4.2.3 pH, Alkalinity and Volatile Fatty Acid (VFA)

Generally, pH, alkalinity and VFA are usually used as operating control parameters and indication the stability of anaerobic wastewater treatment. Measuring effluent pH is a simply control parameter for anaerobic system. When combined with VFA can help indicate the imbalance of system more accurately. If the VFA production rate exceeds the maximum consume capacity of methanogens, the accumulation of excess VFA will begin and consequently lower pH value in the system.

Figure 4.4 illustrates profiles of the effluent pH, alkalinity, and VFA of the 3C-OLR4, 6C-OLR4, and 8C-OLR4 experiments. The effluent pH and alkalinity values were around 7.9 and 2,000 mg/l as $CaCO_3$, respectively (values in detail were shown in Table 4.3). The fluctuation of the three parameters was mostly insignificant for overall experimental period in all ABRs. However adjusting HRT, especially lowering HRT affected on pH and alkalinity concentration, but only a small drop which could be recovered within a few days. It should be note that when OLR increased (decreasing HRT) the system obtained more substrate consequently, more VFA production occurred.

The effluent VFA concentrations during overall the experimental period were monitored and mostly were less than 500 mg/l as $CaCO_3$, which indicated that the stability of the system in substrate consumption. The observation from Figure 4.4 illustrated that every increase in OLRs (or decreasing HRT) induced a temporary increasing VFA. Those were because of higher OLR resulted in the higher production of VFA (Grady et al., 1999; Nachiyasit and Stuckey, 1995; Langenhoff et al., 2000; Akunna and Clark, 2000). However, effluent VFA/alkalinity ratio of all ABRs was maintained below the value of 0.4, which indicated that system had high buffer capacity (Behling et al., 1997). The VFA/alkalinity ratios at steady-state of the 3C-OLR4, 6C-OLR4, and 8C-OLR4 experiments were 0.2, 0.17, and 0.2, respectively (Table 4.3).

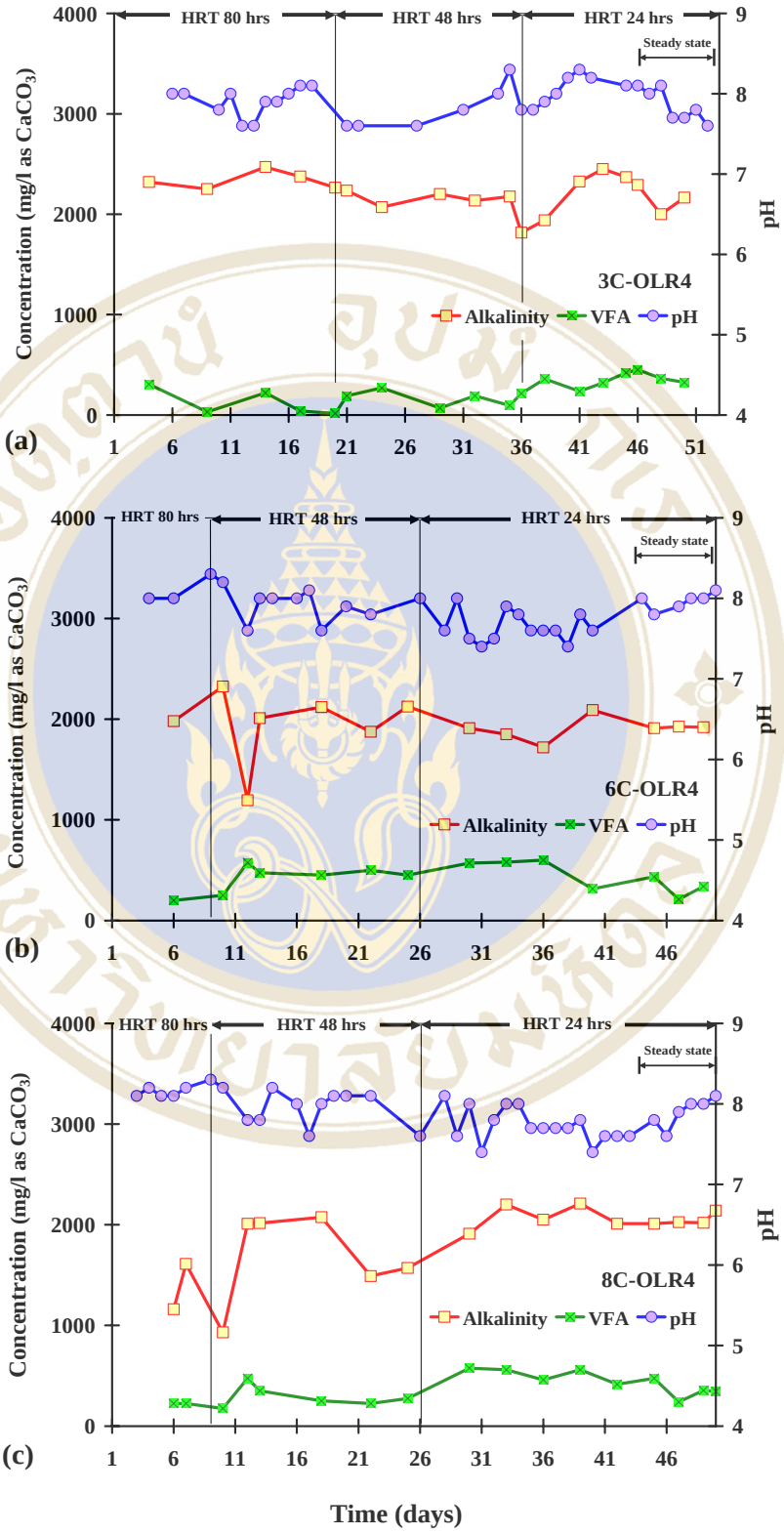


Figure 4.4 Overall effluent pH, alkalinity and VFA of the experiments; (a) 3C-OLR4 (b) 6C-OLR4 (c) 8C-OLR4.

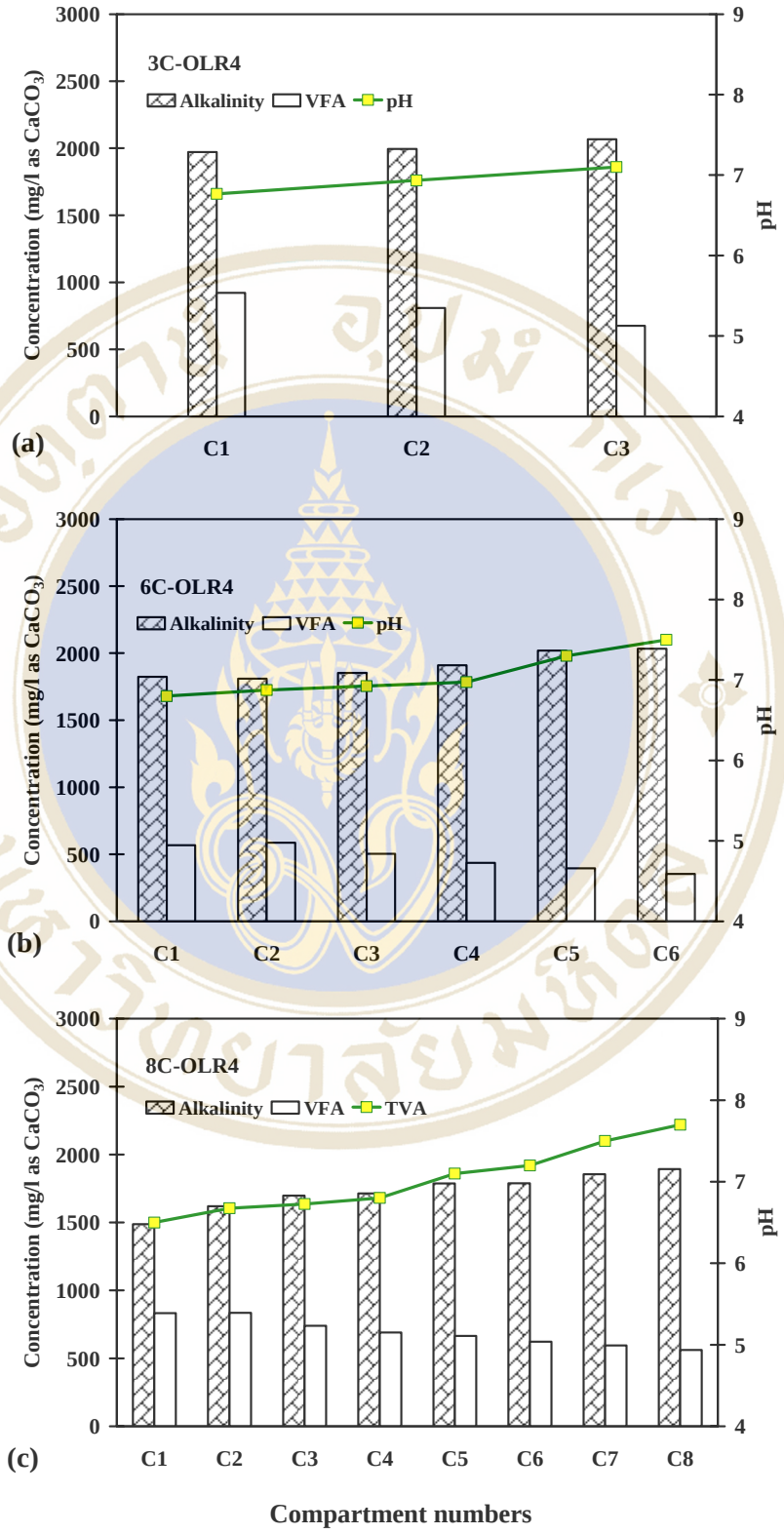


Figure 4.5 pH, alkalinity, and VFA profiles in each compartment numbers during steady-state of the experiments; (a) 3C-OLR4 (b) 6C-OLR4 (c) 8C-OLR4.

Figure 4.5 shows the pH, alkalinity and VFA profiles in each compartment during steady-state. The pH was observed to increase lengthwise through the reactor, which verified the assumptions of microbial phase separation could be occurred in ABR (Nachiyasit and Stuckey, 1995). The front of reactor acts like an acidogenic phase, which favorable pH was around 5.5-6.5, where as the last of reactor performs as methanogenic phase, which most of pH value were around 7.8–8.2. The data of pH, alkalinity, and VFA concentrations in each compartment was summarized in Table 4.4.

However, a phase separation was not obvious in the 3C-OLR4 experiments where little different pH occurred among the three compartments (6.8, 6.9, and 7.1). In the experiment of 6C-OLR4 and 8C-OLR4, phase separations were more evident, especially in consideration of pH value. That is, pH values in the first and the last compartment of the 8C-OLR4 experiment were 6.8 and 7.5 and were 6.5 and 7.7 in the experiment of 8C-OLR4, respectively. It appears that more compartments in a reactor could induce the proper environment for two phase anaerobic operation. In order to confirm this statement, the FISH technique was used to study microbial distribution throughout the reactor. The details were described in Topic 4.4.

Furthermore, Figure 4.5 illustrates that VFA concentration also decreased longitudinally down the reactor. In the first compartment, acidogenic bacteria utilized substrate and convert into intermediate products, such as VFA. Consequently, high VFA accumulation occurred, then, methanogenic bacteria (predomainnant organism group in latter compartments) converted these VFA to methane gas, resulting in lengthwise decreasing VFA through the reactor (Azbar et al., 2001).

4.2.4 Oxidation-Reduction Potential (ORP)

An ORP values can be used to reflect the reaction in biological wastewater treatment. Figure 4.6 demonstrated ORP in each compartment of the ABR during steady-state. It can be seen that, ORP values among the compartment were not significantly different, i.e. the ORP was in the range of -300 to -400 mV (Table 4.4)

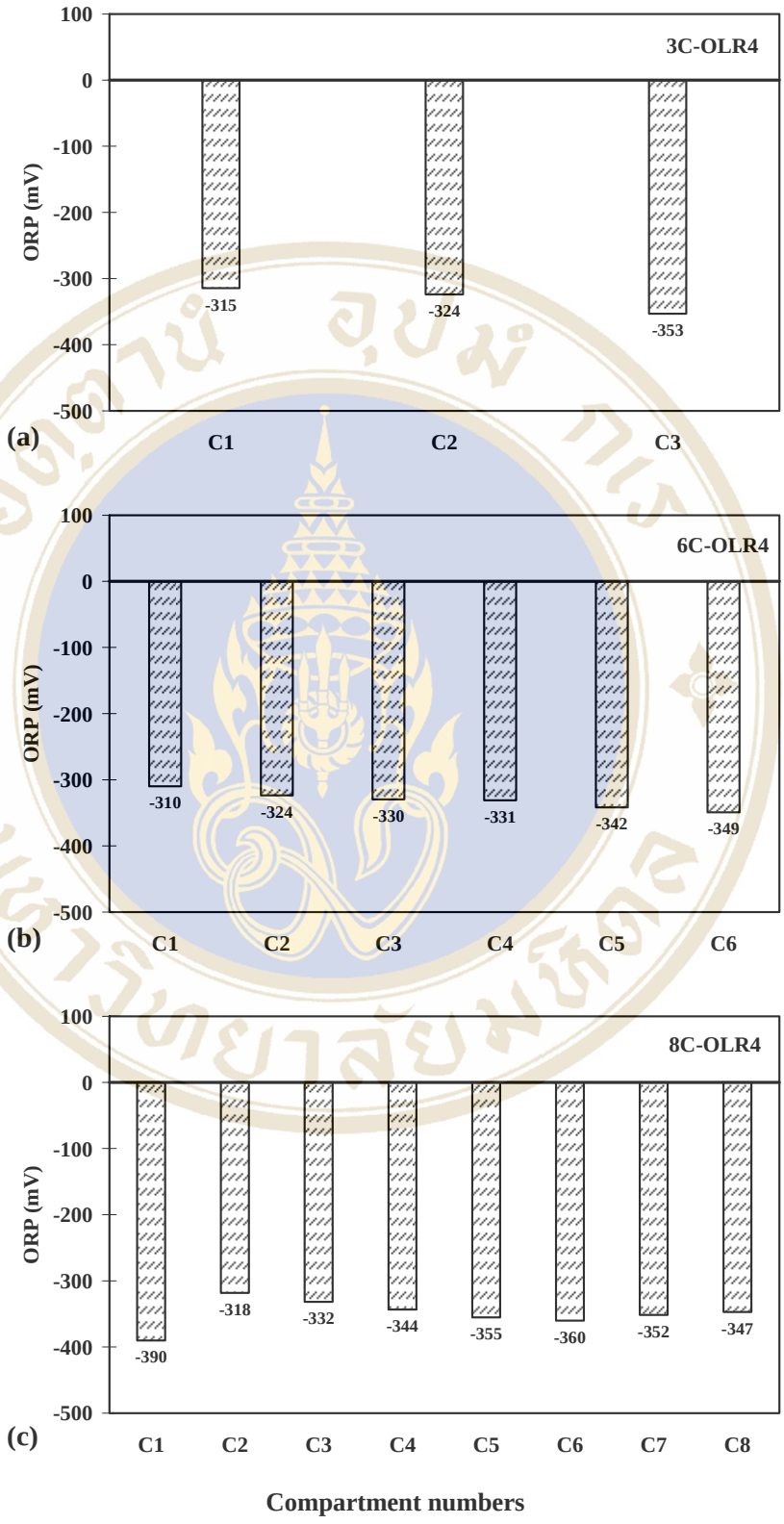


Figure 4.6 ORP values in each compartment during steady-state of the experiments; (a) 3C-OLR4 (b) 6C-OLR4 (c) 8C-OLR4.

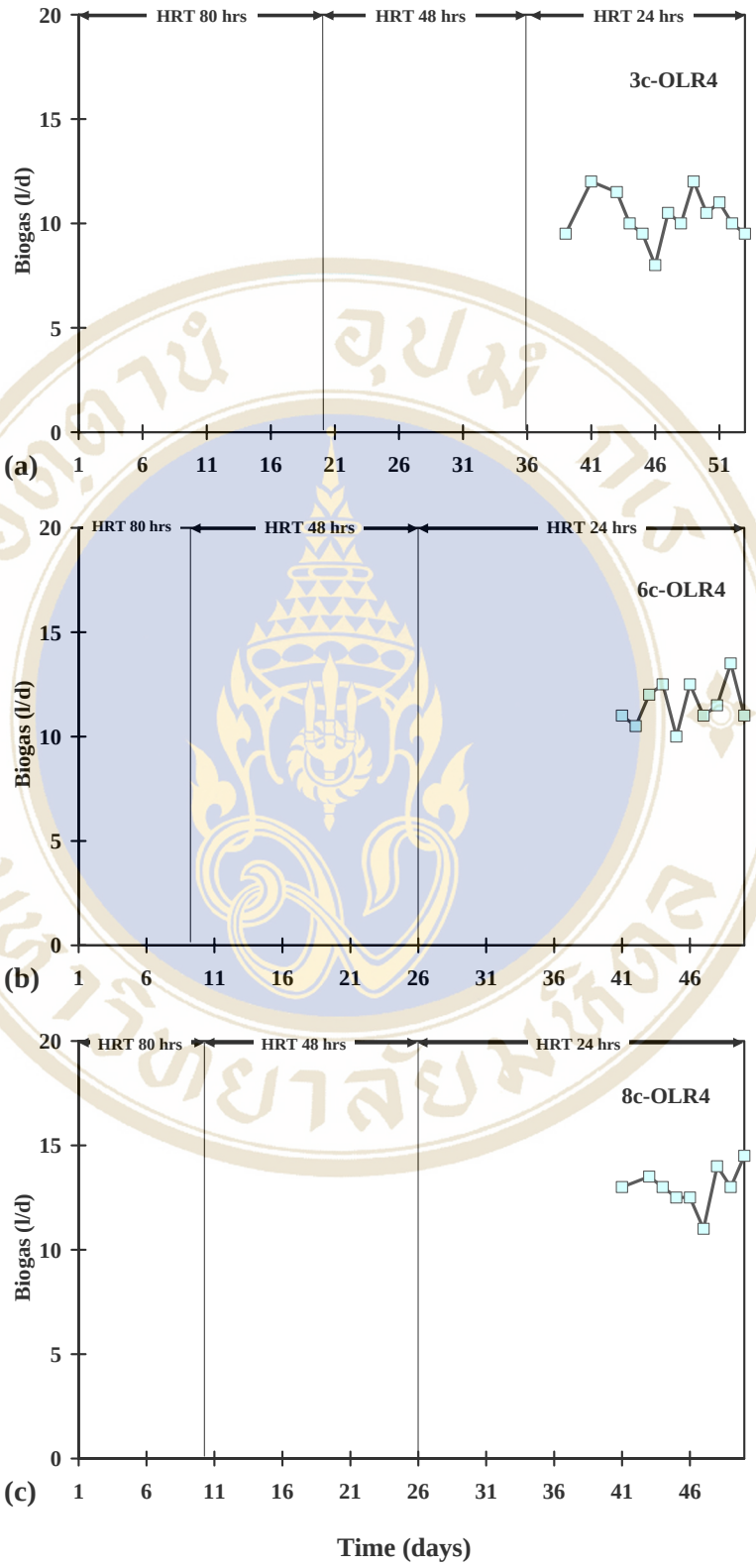


Figure 4.7 Biogas production rate of the experiments;
(a) 3C-OLR4 (b) 6C-OLR4 (c) 8C-OLR4.

which indicated strictly a anaerobic condition occurred within the ABR start from the first compartment.

4.2.5 Biogas Production

During the steady state, an average biogas production rate of the experiment of the 3C-OLR4, 6C-OLR4, and 8C-OLR4 were 10, 12, and 13 l/d, respectively. It appears that biogas production rates among the three experiments were not evidently different as OLR were all the same. At firstly experiment, the reactor lid had leaked a biogas subsequent in loss data on the biogas volume at these times (Figure 4.7).

However, a slightly higher biogas production rate seemed to happen in the ABR with more compartments. This occurrence needs further investigation to confirm this observation.

4.2.6 Solid Retention Time (SRT)

Figure 4.8 depicts the effluent solid (TSS and VSS) concentrations in every experiment of this part. The average data of TSS and VSS concentration during steady-state were presented in Table 4.3.

In the experiment of 3C-OLR4 (Figure 4.8a), the effluent solid concentration were quite high, especially during the start-up and decreasing HRT period, which the effluent solid concentrations occasionally reached 2,000–3,000 mg TSS/l. In the 6C-OLR4 experiment, an effluent solid concentration during start-up period was lower than 300 mg TSS/l (Figure 4.8b). Nonetheless, high effluent solid concentrations 1,500-1,800 mg TSS/l found during the operation with HRT of 48 hrs and again after 24 hrs HRT was adjusted. The effluent solid concentrations of the 8C-OLR4 experiment were obviously low (200-300 mg TSS/l) in comparison with the other experiments (Figure 4.8c). The highest effluent solid concentration (less than 1,000 mg TSS/l) was found during changing of operating HRT from 48 to 24 hrs. When each experiment finished, residual solids in every compartment of the reactors were

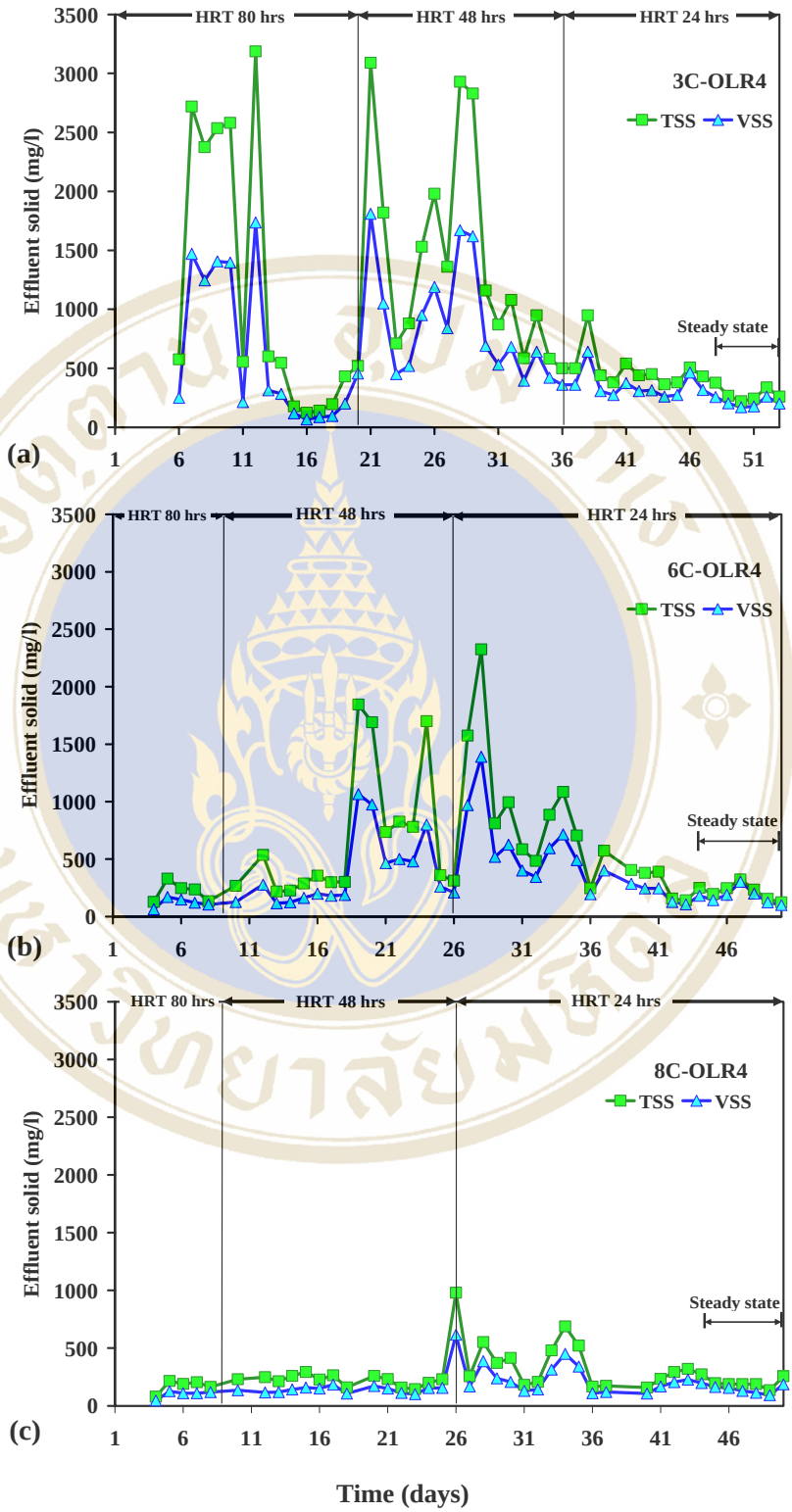


Figure 4.8 Overall effluent solid concentration of the experiments; (a) 3C-OLR4 (b) 6C-OLR4 (c) 8C-OLR4.

rinse out, and measured sludge concentration and total biomass. Then, SRT of each ABRs was calculated according to equation 1.1.

The results of solid were calculated and summarized in Table 4.5, which values were derived from laboratory analysis and calculation by equation 1.1 and 4.1-4.6. Figure 4.9 show sludge balance in the first experiment based on inoculated sludge concentration controlled to be the same of 28 g TSS/l. At the end of the experiments, final sludge was 99, 159, and 257 g TSS resided in the 3C-OLR4, 6C-OLR4, and 8C-OLR4 experiments, respectively. Total sludge washout were 243, 190, and 94 g TSS, respectively, while observed yield were 0.02, 0.03, and 0.03 g VSS/g COD, respectively.

Sludge produced in the experiment 3C-OLR4 (20 g VSS) was significant lower than those in the experiments of 6C-OLR4 and 8C-OLR4 (38 and 40 g VSS, respectively). It could be explained that each compartment in the three-compartment reactor larger than those in the six-compartment and the eight-compartment reactors, more dead-space could occur and reduce the contact between sludge and substrate. Also, the mixing in the six-compartment and the eight-compartment reactors from the visual observation was quite good. Dama et al. (2002) recommend that the ratio of up-flow and down-flow width of 1:3 helped reduce a dead-space occurrence. However, the dead space was still occurred in the three-compartment reactor used in this study through the ratio of 1:3 was established.

As mention above, one may conclude from these experiments that the number of compartment has affected on maintainability of sludge within ABRs, the more compartment numbers, the lower sludge washout rate is and the higher sludge resides in the reactor. Apparently, with more compartment numbers obtained higher SRT and SRT/HRT ratio, i.e. the SRT and SRT/HRT ratio were 35, 74, and 134 days and the SRT/HRT ratio were 35, 74, and 134 d/d for the experiments of 3C-OLR4, 6C-OLR4, and 8C-OLR4, respectively (Figure 4.10). In this study, sludge yield, in according to the equation 4.6, were affected by compartment numbers because both of sludge production and COD utilization concurrently increased.

$$\text{SRT (d)} = \frac{\text{Sludge in reactor (g)}}{\text{Sludge washout rate (g/d)}} \quad (1.1)$$

$$\text{Initial sludge (g)} = \text{Seed sludge concentration (g/l)} \times \text{reactor volume (l)} \quad (4.1)$$

$$\text{Final sludge (g)} = \text{Total sludge in reactor (g/l)} \times \text{reactor volume (l)} \quad (4.2)$$

$$\text{Sludge washout rate (g/d)} = \text{Effluent TSS (g/l)} \times \text{flow rate (l/d)} \quad (4.3)$$

$$\text{Total sludge washout (g)} = \text{Sludge washout rate (g/d)} \times \text{Operation time (d)} \quad (4.4)$$

$$\text{Sludge produce (g)} = \text{Final sludge (g)} - \text{Initial sludge (g)} + \text{Total sludge washout (g)} \quad (4.5)$$

$$\text{Yield (g VSS/g COD)} = \frac{\text{Sludge produce (g VSS)}}{\text{Total substrate utilize (g COD)}} \quad (4.6)$$

Note: Eq.1.1 = Sludge in reactor is the Final sludge (g TSS).
Eq.4.3 = Averaging sludge washout rate during steady-state.

Table 4.6 presents the SRT and SRT/HRT ratios from this study in comparison with other anaerobic systems, especially CSTR and UASB. When consider a conventional anaerobic digestion that usually is continuous flow stirred tank reactor (CSTR), its HRT mostly is the same as SRT, resulting in the ratio of SRT/HRT=1. The ABR system used in this study and other works (listed in Table 4.6) was found to provide the SRT/HRT ratio very much higher than a conventional anaerobic digestion. The SRT and SRT/HRT ratios achieved from this study were similar to the study of Grobicki and Stuckey (1991) using the same numbers of compartment reactor. From the studies of Torkian et al. (2003) and Singh and Viraraghavan (2003), the UASB obviously achieved higher SRT/HRT ratio than the ABR system, but the complexity of reactor design and operation may be incomplete. Nonetheless SRT and SRT/HRT ratios of ABRs achieved from the studies of

Boopathy (1998), and Boopathy and Sievers (1991) were very low, when treating swine wastewater, which contained high solid content. In addition to their lower biodegradability, wasting sludge to prevent clogging was necessary, resulting in lower SRT and SRT/HRT ratios.

Table 4.5 The details of sludge in the experiments of Part I.

Parameters	**Eq.**	**3C-OLR4**		**6C-OLR4**		**8C-OLR4**	
		TSS	***VSS***	***TSS***	***VSS***	***TSS***	***VSS***
Initial sludge (g)	*(4.1)*	288	196	273	211	276	216
Final sludge (g)	*(4.2)*	99	67	159	127	257	193
Sludge washout rate (g/d)	*(4.3)*	2.8	2.1	2.1	1.8	1.9	1.4
Total sludge washout (g)	*(4.4)*	243	149	190	121	94	62
Sludge produce (g)	*(4.5)*	54	20	75	38	75	40
Yield (g VSS/ g COD)	*(4.6)*	0.02		0.03		0.03	
SRT (days)	***(1.1)***	**35**		**74**		**134**	

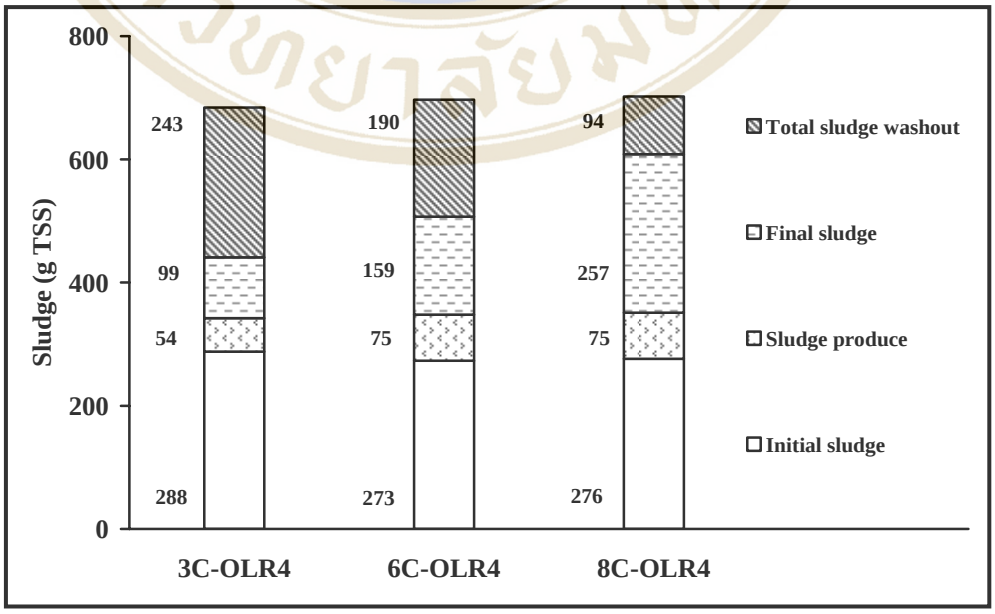


Figure 4.9 Sludge balance in the experiments of Part I.

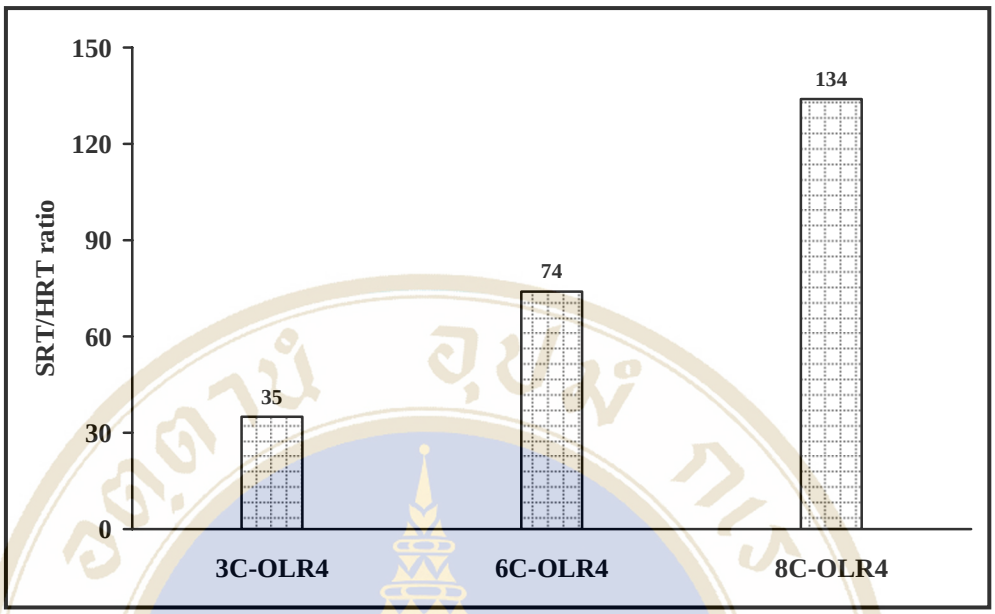


Figure 4.10 The SRT/HRT ratios in consideration of various compartment numbers.

4.2.6 Sub-conclusion

In conventional anaerobic reactor with an SRT/HRT ratio of 1, there is some limitation in treating high strength wastewater due to slow growth rate of anaerobic microorganism. Since wash out of anaerobic sludge is the only cause of lowering SRT unintentionally, many solutions, such as returning sludge, using a filter for trapping the biomass or even packing some media in the reactor, were endeavor. However, the cost of filter and packing material is the weakness. Moreover, the problems of clogging, more pumping power required or the need of the packing level control and wasting with ingrowths were much concerned.

The compartmentalized structure of ABR can retard sludge washout rate without additional equipments and complicated operation. Compartments numbers is a significant factor influence on SRT and SRT/HRT ratio that high compartment numbers affected higher SRT and SRT/HRT ratio. The result from this study concluded that eight-compartment ABR obtained both higher reactor performance and SRT and SRT/HRT ratio than three-compartment and six-compartment ABRs. **Hence, the optimum SRT/HRT ratio based on this study was 134 day/day which achieved from eight-compartment ABR which OLR of 4 g COD/l-d.**

Table 4.6 Comparison of SRT and SRT/HRT ratios with other related studies.

Reactor	Wastewater	No.[a]	HRT (days)	SRT (days)	SRT/HRT ratio	References
Low solid content wastewater						
CSTR					1	
ABR	Carbohydrate-protein	**3**	**1**	**35**	**35**	**This study**
ABR	Carbohydrate-protein	4	0.8	42	50	Grobicki and Stuckey (1991)
ABR	*p*-nitrophenol	4	1	48	48	Kuscu and Sponza (2006)
ABR	Carbohydrate-protein	6	0.8	60	72	Grobicki and Stuckey (1991)
ABR	Carbohydrate-protein	**6**	**1**	**74**	**74**	**This study**
ABR	Carbohydrate-protein	8	0.8	70-106	84-128	Grobicki and Stuckey (1991)
ABR	Carbohydrate-protein	**8**	**1**	**134**	**134**	**This study**
UASB	Slaughterhouse	-	0.09-0.3	3.3-60.3	34-203	Torkian et al. (2003)
UASB	Municipal	-	0.2	79-183	447-1,076	Singh and Viraraghavan (2003)
High solid content wastewater						
ABR	Swine manure	2	15	22	1.5	Boopathy and Sievers (1991)
ABR	Whole swine waste	2	14	25	1.8	Boopathy (1998)
ABR	Swine manure	3	15	25	1.7	Boopathy and Sievers (1991)
ABR	Whole swine waste	3	14	30	2.1	Boopathy (1998)
ABR	Whole swine waste	4	14	36	2.6	Boopathy (1998)
ABR	Whole swine waste	5	14	42	3	Boopathy (1998)

[a] Compartment numbers

4.3 Part II: Optimum Organic Loading Rate

The aim of this part was to investigate the optimum OLR of ABR for treating carbohydrate-protein wastewater. From the results in the previous part, the ABR consisted of eight compartments (8C) was selected as it achieved the highest performance and SRT and SRT/HRT ratio.

4.3.1 Wastewater Characteristic

Wastewater used in this part was daily prepared following the substances listed in Table 3.1. To obtain the COD concentrations of 4,000, 8,000, 12,000, and 16,000 mg/l, only sucrose and nutrient broth in Table 3.1 were multiplied by 1, 2, 3 and 4, respectively. Wastewater characteristic used in this part was shown in Table 4.7.

Influent COD concentrations in the experiment of 8C-OLR4, 8C-OLR8, 8C-OLR12, and 8C-OLR16 were averaged at 4,230, 8,300, 12,463 and 16,301 mg/l, respectively. The average influent pH was of 8.1, 8.3, 8.3, and 8.5, and alkalinity were of 2,040, 2,148, 1,963, and 2,606 mg/l as $CaCO_3$, respectively.

The prepared COD concentration, pH, and alkalinity in this part of experimental were mostly in the designated level which was satisfactory to maintain the reactor performance. However the experiment of 8C-OLR16 was exempt, particularly its pH and alkalinity that were higher than other experiments. The addition of more sodium bicarbonate in order to maintain pH buffering capacity was done due to pH decrease occurred in this experiment. The decrease of pH happened concurrently with the increase of VFA accumulation due to very high OLR (16 g COD/l-d) was applied.

4.3.2 The Overall COD Removal Efficiency

The overall COD removal efficiencies of every experiment in this part show in Table 4.7. From Figure 4.11a the experiment of 8C-OLR4 was operated with 80 hrs HRT

Table 4.7 Performance of the ABRs during steady-state in Part II.

Parameters		8C-OLR4		8C-OLR8		8C-OLR12		8C-OLR16	
		Inf [c]	*Eff* [d]	*Inf*	*Eff*	*Inf*	*Eff*	*Inf*	*Eff*
pH	*Mean*	8.1	7.9	8.3	8.4	8.3	8.1	8.5	7.2[f]
	SD.	*0.25*	*0.20*	*0.2*	*0.1*	*0.1*	*0.1*	*0.14*	*0.26*
tCOD[a]	*Mean*	4,230	1,335	8,300	585	12,463	2,290	16,301	*11,467*[f]
(mg/l)	*SD.*	*126.5*	*59.0*	*190.5*	*85.3*	*240.1*	*200.4*	*161.0*	-
sCOD[b]	*Mean*		731		370		1,557	[f]	*10,667*[f]
(mg/l)	*SD.*		*17.2*		*47.7*		*210.1*		-
COD removal	*Mean*		83		96		88		34[f]
efficiency *(%)*[e]	*SD.*		*0.8*		*0.6*		*1.7*		-
COD utilization	*Mean*		25.23		79.3		109		-[f]
rate *(g/d)*	*SD.*		*11.8*		*2.1*		*3.29*		-[f]
TSS	*Mean*		203.4		294.3		699		1,030
(mg/l)	*SD.*		*46.1*		*39.6*		*43.9*		447.9
VSS	*Mean*		148.8		212.4		496		742.5
(mg/l)	*SD.*		*40.0*		*47.3*		*72.3*		363.7
Slugde washout	*Mean*		2.0		2.9		6.99		10.3
rate *(g TSS/d)*	*SD.*		*4.6*		*0.40*		*0.44*		*4.48*
Alkalinity	*Mean*	2,040	2,048	2,148	2,407	1,963	2,472	2,606	2,590[f]
(mg/l as $CaCO_3$)	*SD.*	*104.9*	*61.1*	*210*	*66.58*	*128.2*	*106.93*	*100.7*	*60.9*
VFA	*Mean*		353		57		551.7		1,683[f]
(mg/l as $CaCO_3$)	*SD.*		*96.1*		*5.8*		*84.3*		*230*
VFA/Alkalinity	*Mean*		0.2		0.02		0.2		-[f]
ratio	*SD.*		*0.05*		*0.0*		*0.04*		-[f]
Biogas	*Mean*		13		40		48		54
volume *(l/d)*	*SD.*		*1.07*		*2.08*		*3.30*		*1.00*

Note: [a] total COD [b] soluble COD

[c] Influent [d] Effluent

[e] $\frac{(\text{tCOD}_{\text{Inf}} - \text{sCOD}_{\text{Eff}}) \times 100}{\text{tCOD}_{\text{Inf}}}$

[f] not steady-state

(or OLR 1.2 g COD/l-d) for about 9 days, COD removal efficiency in this period was averaged at 91%. Then, HRT was decreased to 48 hrs (or OLR 2 g COD/l-d), COD removal efficiency was around 83%, and at steady-state of 24 hrs HRT operation (or 4 gCOD/l-d), an average COD removal efficiency was 83%.

A soluble COD (sCOD) removal efficiency in the experiment 8C-OLR8 was quite constant throughout the operation period, and mostly was higher than 95%. While the percentage of total COD (tCOD) removal efficiency was sometime occasionally dropped in accordance with high effluent solid (see Figure 4.11b).

During the early period of the 8C-OLR12 experiment (HRT of 80 hrs or OLR 4.8 g COD/l-d), COD removal efficiency was achieved around 75%, and then increase to 94% though the operating HRT was adjusted to 48 hrs (or OLR 6 g COD/l-d). At steady-state of 24 hrs HRT operation (or OLR 12 g COD/l-d) the COD removal efficiency was averaged at 88% (Figure 4.11c).

In this part, the inoculated sludge for the 8C-OLR16 experiment was obtained from the experiment of an 8C-OLR8 (8 g COD/l-d), therefore, sludge was already to carbohydrate-protein wastewater. In order to reduce the start-up period, the 8C-OLR16 experiment was initially operated with designated HRT of 24 hrs and influent COD concentration of 8,000 mg/l (8 g COD/l-d), nevertheless, the COD removal efficiency observed during the first week continuously decreased, This could be said that, the system might not have adequate capability for starting up with initial OLR 8 g COD/l-d. Nachaiyasit (1995) reported that start-up with initial high OLR (13 kg COD/m^3-d) occur the accumulation of intermediate products causing reactor souring and eventual failure after two weeks of operation (Barber and Stuckey, 1999). Hence, start-up with initial high OLR was not recommended though inoculated sludge was acclimated. The OLR, then, was decreased to 4 g COD/l-d by increased HRT to 48 hrs, and apparently, the COD removal efficiency gradually increased to about 90%. Next, OLR was increased to 6 g COD/l-d (by keep constant 48 hrs HRT, but decreased COD concentration to 12,000 mg/l), the removal efficiency decreased to around 85%. When increase OLR to 8 g COD/l-d (by keep constant 48 hrs HRT, but

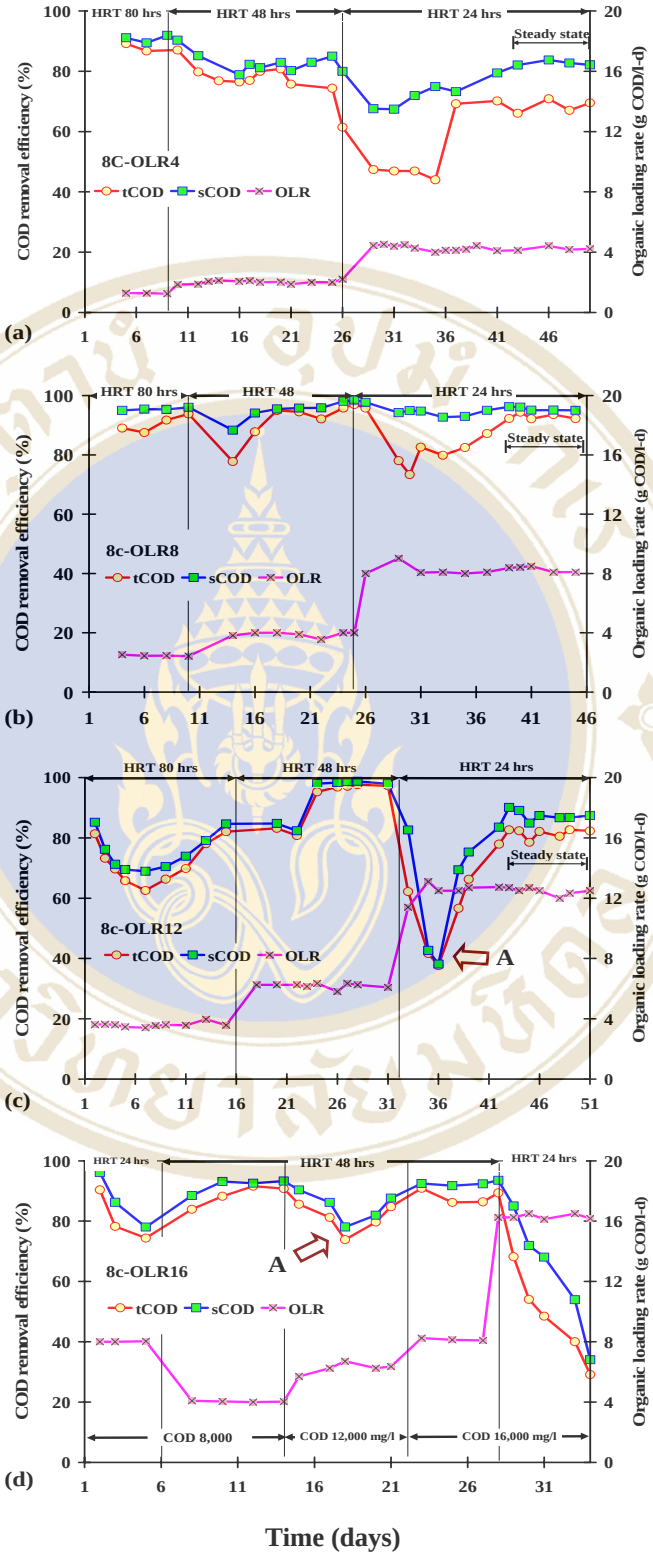


Figure 4.11 Overall COD removal efficiency and organic loading rate of the experiments; (a) 8C-OLR4 (b) 8C-OLR8 (c) 8C-OLR12 (d) 8C-OLR16.

COD concentration increased to 16,000 mg/l), the COD removal efficiency decreased to at 90%. Then, the OLR was increased to the designated one on 16 g COD/l-d by decrease HRT to 24 hrs, the COD removal efficiency rapidly decreased. Within five days after increasing OLR to 16 g COD/l-d, the COD removal efficiency was down to 34% (Figure 4.11d). In additional, the tCOD and sCOD removal efficiencies of eight-compartment various ABRs in this experiment part were not much different because the compartmentalized structure could retain sludge within the reactor quite well, resulted in low sludge washout.

The failure of the experiment 8C-OLR16 could no evidently blame on the high OLR of 16 g COD/l-d yet. It is possible that OLR was increased too much and too fast the system could not be tolerated.

It is well known that, anaerobic microorganism are sensitive with a small environmental changes such as temperature. The low temperature, the lower microbial activity occurs. The sudden reduction of COD removal efficiency, the increasing of effluent VFA (due to reduce activity of methanogens), and decrease effluent pH, were found during the experiments of 8C-OLR12 and 8C-OLR16. At the moment of this occurrence (marked by A symbol in Figure 4.11c and 4.11d), it was during the month of December 2006, which was the winter season in Thailand. The water temperature was down from normal temperature of about 29-30 degrees Celsius to around 20-23 degrees Celsius. During this occurrence, the influent feeding was stopped for three days for prevent shock loading, and light bulb was used to heat the reactor. With this manipulation, the removal efficiency was gradually increased to about 90% in both ABRs because the water temperature was raise almost close to the normal range (27-28 degrees Celsius).

Figure 4.12 depicts the profiles of COD concentration and the removal efficiency in each compartment of ABRs with various OLRs. It was seen that, almost fifty percents of the COD concentration was since in the first compartment, and the COD removal efficiency increased longitudinally from the inlet through the outlet. The 8C-OLR4 experiment, COD concentration decreased from 4,000 mg/l influent to

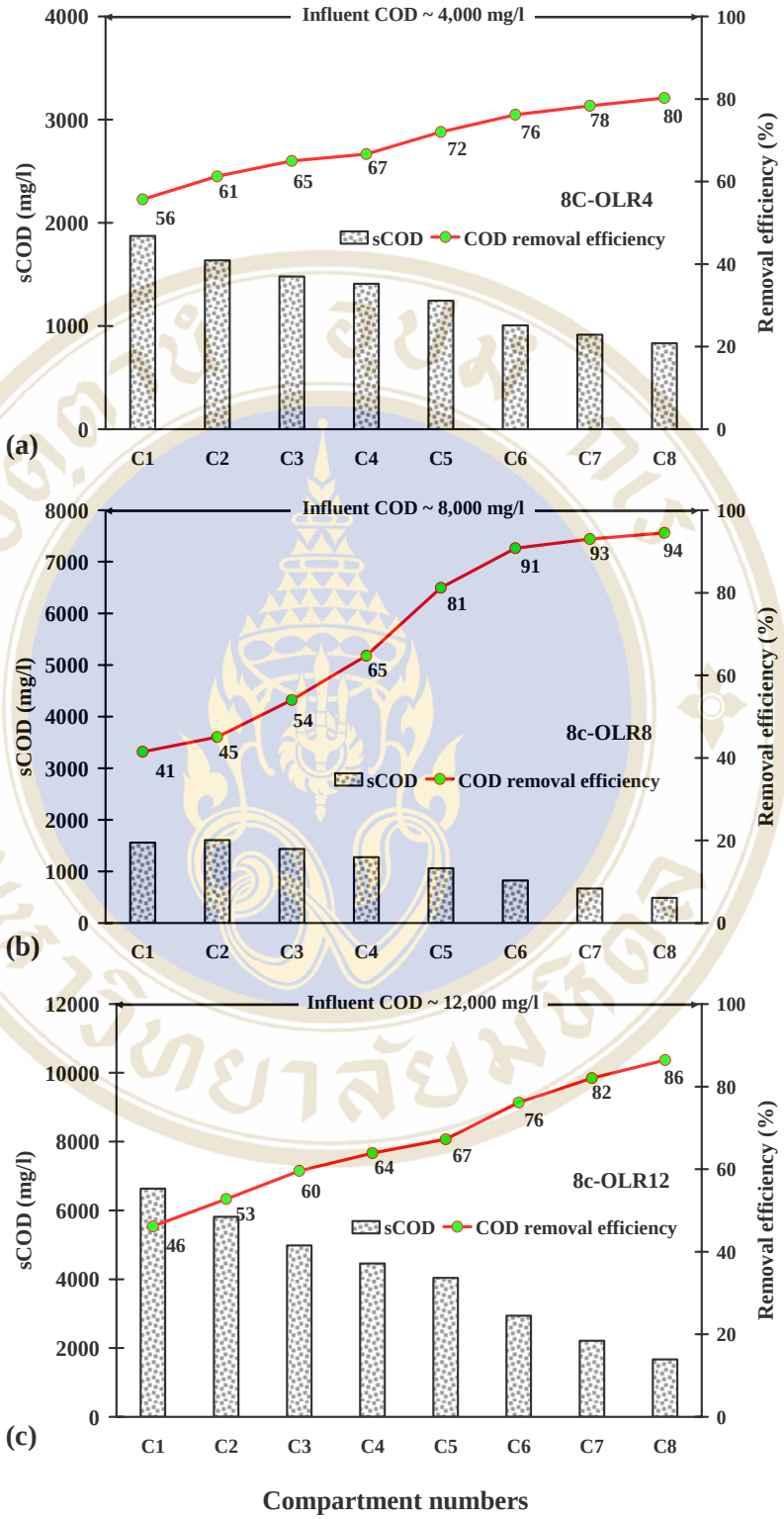


Figure 4.12 COD profiles in each compartment numbers during steady-state of the experiment; (a) 8C-OLR4 (b) 8C-OLR8 (c) 8C-OLR12

about 1,800 mg/l (55% removal efficiency) in the first compartment (C1), then, the COD continuously decreased longitudinally and achieved 80% removal efficiency in the eighth compartment (C8) (see Figure 4.12a). In the 8C-OLR8 experiment, the COD concentration decreased approximately from 8,000 mg/l to 4,800 mg/l in the first compartment (about 40% removal efficiency) while 94% removal efficiency was achieved in the eighth compartment (C8) as shown in Figure 4.12b. In the first compartment of the 8C-OLR12 experiment, about 45% of COD removal occurred (decreased from 12,000 to 6,600 mg/l), and achieved 85% of the removal efficiency in the eighth compartment (C8) (Figure 4.12c). The details of COD concentrations and the removal efficiencies of ABRs in each compartment were presented in Table 4.8, except those of the 8C-OLR16 experiment due to its failure to reach the steady-state.

4.3.3 pH, Alkalinity and Volatile Fatty Acid (VFA)

Overall effluent pH, alkalinity, and VFA profiles were illustrated in Figure 4.13. It appeared that effluent pH and alkalinity values throughout the operation time of all every ABRs was almost constant. The average effluent pH values of 8C-OLR4, 8C-OLR8, and 8C-OLR12 experiment during steady-state were around 8.0. Effluent VFA/alkalinity ratio of every ABRs was less than 0.4 which indicated that ABR still had enough buffering capacity to maintain constant pH value in the system (Behling, 1997). Surprisingly, alkalinity value continued increasing during the first fifteen days of operation in 8C-OLR8 (almost reached 4,000 mg/l as $CaCO_3$) with unknown reason, however, it gradually decreased and was almost constant around 2,400 mg/l as $CaCO_3$ and stable until steady-state (Figure 4.13b). Average pH, alkalinity, and VFA of ABRs in this part were also shown in Table 4.7.

In this study, Sodium bicarbonate ($NaHCO_3$) of 3,000 mg/l was added to the influent to supply alkalinity of pH buffering capacity to the system. Interestingly, the effluent pH and alkalinity of all ABRs both in Part I and Part II, expect in the experiment of 8C-OLR16, slightly higher than 8.0 and 2,000 mg/l as $CaCO_3$, respectively, and VFA/alkalinity ratio was very low (about 0.2). It signifies that less buffering could be required in ABR through OLR increased up to 12 g COD/l-d. Therefore, a smaller amount of $NaHCO_3$ may be adequate for maintaining the ABR performance, resulting in chemical cost reduction.

Table 4.8 Average profile performance at steady-state of ABRs in the Part II experiment.

	No.[a]	pH	sCOD (mg/l)	Efficiency (%)	Alkalinity (mg/l as $CaCO_3$)	VFA (mg/l as $CaCO_3$)	VFA/ Alk.	ORP (mV)	TSS (mg/l)
8C-OLR4	C1	6.5	1,873	56	1,487	833	0.6	-390	27,867
	C2	6.7	1,637	61	1,620	835	0.5	-318	23,833
	C3	6.7	1,480	65	1,698	739	0.4	-332	23,867
	C4	6.8	1,410	67	1,713	690	0.4	-344	22,767
	C5	7.1	1,245	72	1,787	665	0.4	-355	21,233
	C6	7.2	1,005	76	1,788	622	0.3	-360	20,433
	C7	7.5	915	78	1,855	595	0.3	-352	17,167
	C8	7.7	834	80	1,893	562	0.3	-347	18,900
8C-OLR8	C1	5.4	4,834	41	1,383	1558	1.1	-340	38,267
	C2	5.7	4,536	45	1,503	1605	1.1	-294	16,763
	C3	6.1	3,799	54	1,755	1435	0.8	-326	21,920
	C4	6.7	2,910	65	1,841	1277	0.7	-342	16,681
	C5	7.0	1,556	81	1,951	1060	0.5	-345	23,572
	C6	7.3	760	91	2,055	825	0.4	-358	21,333
	C7	7.6	576	93	2,185	670	0.3	-362	20,148
	C8	7.8	457	94	2,293	490	0.2	-365	18,258
8C-OLR12	C1	5.7	6,637	46	1,535	1,910	1.2	-374	42,867
	C2	6.3	5,818	53	1,970	1,840	0.9	-358	32,833
	C3	6.5	4,983	60	2,020	1,600	0.8	-321	18,483
	C4	6.7	4,457	64	2,544	1,513	0.6	-335	19,235
	C5	6.7	4,039	67	2,140	1,170	0.5	-342	29,532
	C6	6.8	2,942	76	2,200	1,130	0.5	-345	27,528
	C7	7.0	2,214	82	2,295	1,125	0.5	-347	25,348
	C8	7.3	1,673	86	2,350	1,090	0.5	-350	37,235

[a] = Compartment numbers

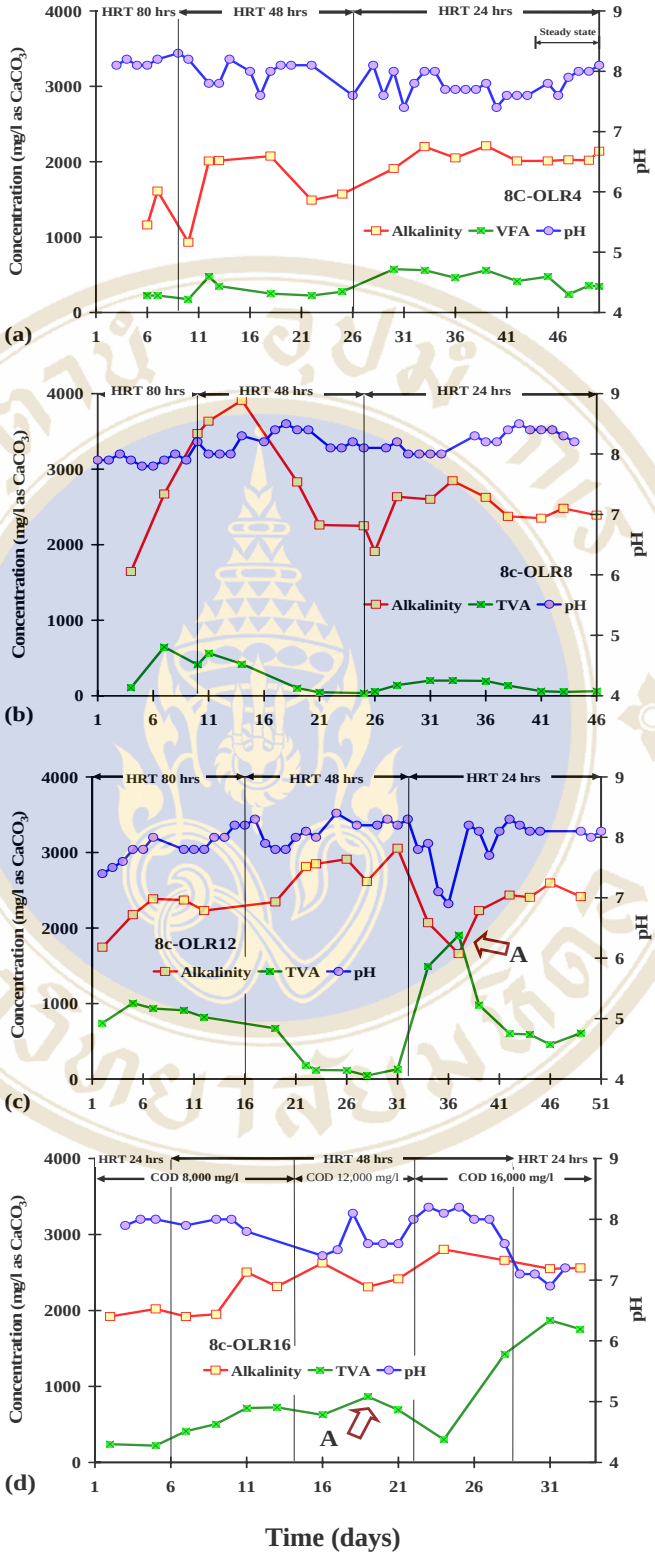


Figure 4.13 Overall effluent pH, alkalinity and VFA of the experiments;
(a) 8C-OLR4 (b) 8C-OLR48 (c) 8C-OLR12 (d) 8C-OLR16.

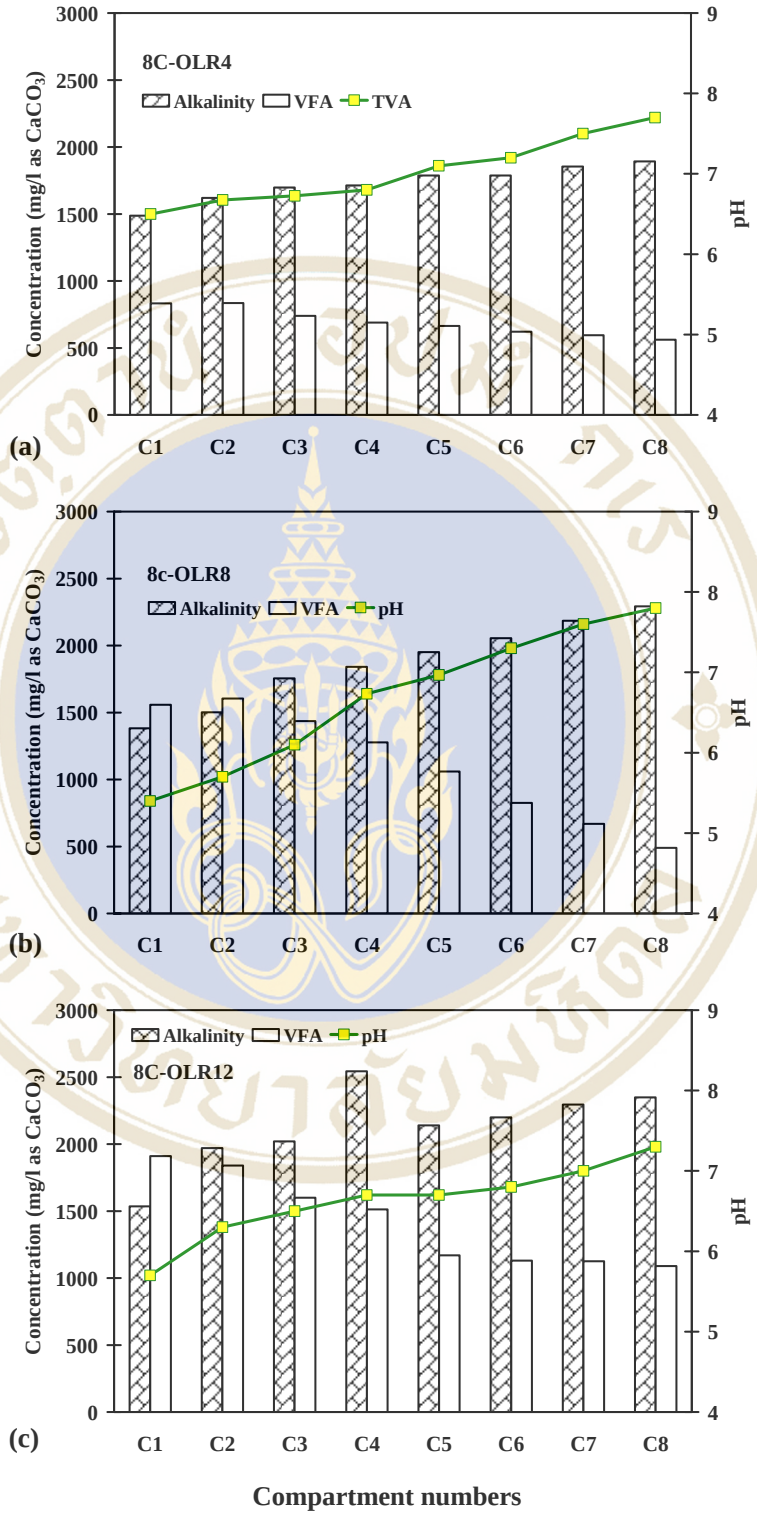


Figure 4.14 pH, alkalinity and VFA profiles in each compartment numbers during steady-state of the experiments; (a) 8C-OLR4 (b) 8C-OLR48 (c) 8C-OLR12.

Effluent VFA concentration of the 8C-OLR4 experiment was averagely lower than 500 mg/l as $CaCO_3$ throughout the operation period (Figure 4.13a). For the experiment of 8C-OLR8, the effluent VFA concentration was about 500-600 mg/l as $CaCO_3$, and then drastically decreased to approximately 50 mg/l as $CaCO_3$. When operated with the OLR of 8 g COD/l-d, VFA concentration gradually increased to about 200 mg/l as $CaCO_3$ during steady-state (Figure 4.13b). VFA concentration in the 8C-OLR12 experiment at the early period of operation was quite high, about 1,000 mg/l as $CaCO_3$. However the VFA concentration decreased to about 100 mg/l as $CaCO_3$ during the operation with HRT of 48 hrs. At steady-state of 24 hrs HRT operation, the VFA concentration of 8C-OLR12 was about 600 mg/l as $CaCO_3$. In this experiment, there was abrupt change in VFA, pH and alkalinity as shown by "A" pointing in Figure 4.13c due to the reduction of ABR performance caused by temperature changing. For the 8C-OLR16 experiment, VFA concentration increased from about 200 mg/l as $CaCO_3$ during start-up period to about 1,800 mg/l as $CaCO_3$ during the operation at OLR of 16 g COD/l-d, which indicated the system imbalance.

The pH, alkalinity, and VFA profiles in each compartment were shown in Figure 4.14 and summarized in Table 4.8. The pH and alkalinity in every ABRs was increased longitudinally from the inlet through the outlet, which in contrast with VFA profiles. The pH in the first compartment of 8C-OLR4, 8C-OLR8, and 8C-OLR12 experiments were 6.5, 5.4, and 5.7, respectively. They increased lengthwise the compartment, and resulted in the last compartment pH were 7.7, 7.8, and 7.3, respectively. It was been demonstrated that, pH in the first compartment of ABRs was appropriated to acidogens, and pH in the last compartment was proper for the methanogens. From this data, it is possible to mention that there was the occurrence of microbial phase separation in ABRs in this study.

4.3.4 Oxidation Reduction Potential (ORP)

ORP profiles in each compartment numbers during the steady-states of 8C-OLR4, 8C-OLR8, and 8C-OLR12 experiments were shown in Figure 4.15. There was not much difference in ORP values among the ABRs experiment in this part, and

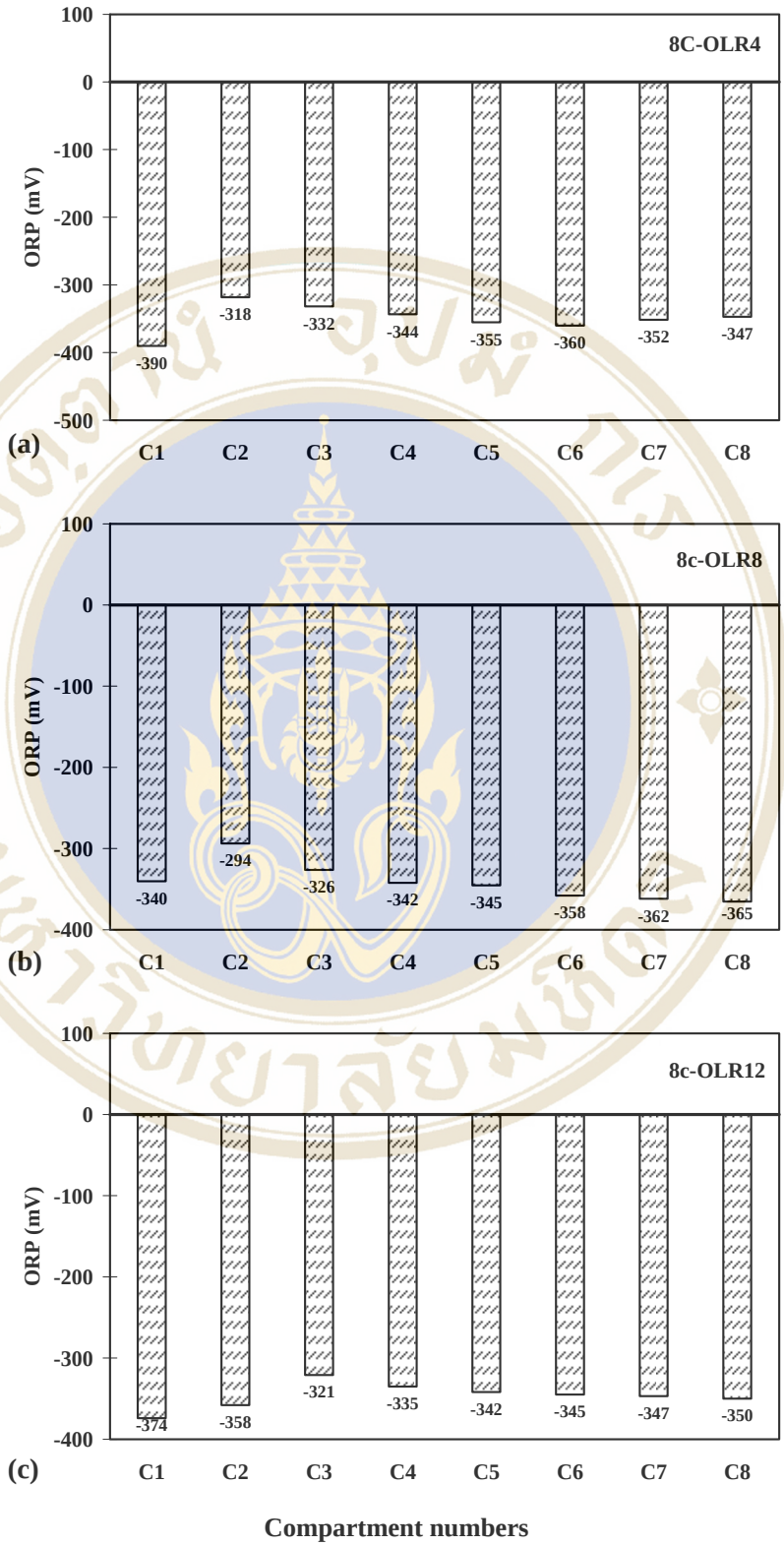


Figure 4.15 ORP profiles in each compartment during steady-state of the experiments; (a) 8C-OLR4 (b) 8C-OLR48 (c) 8C-OLR12.

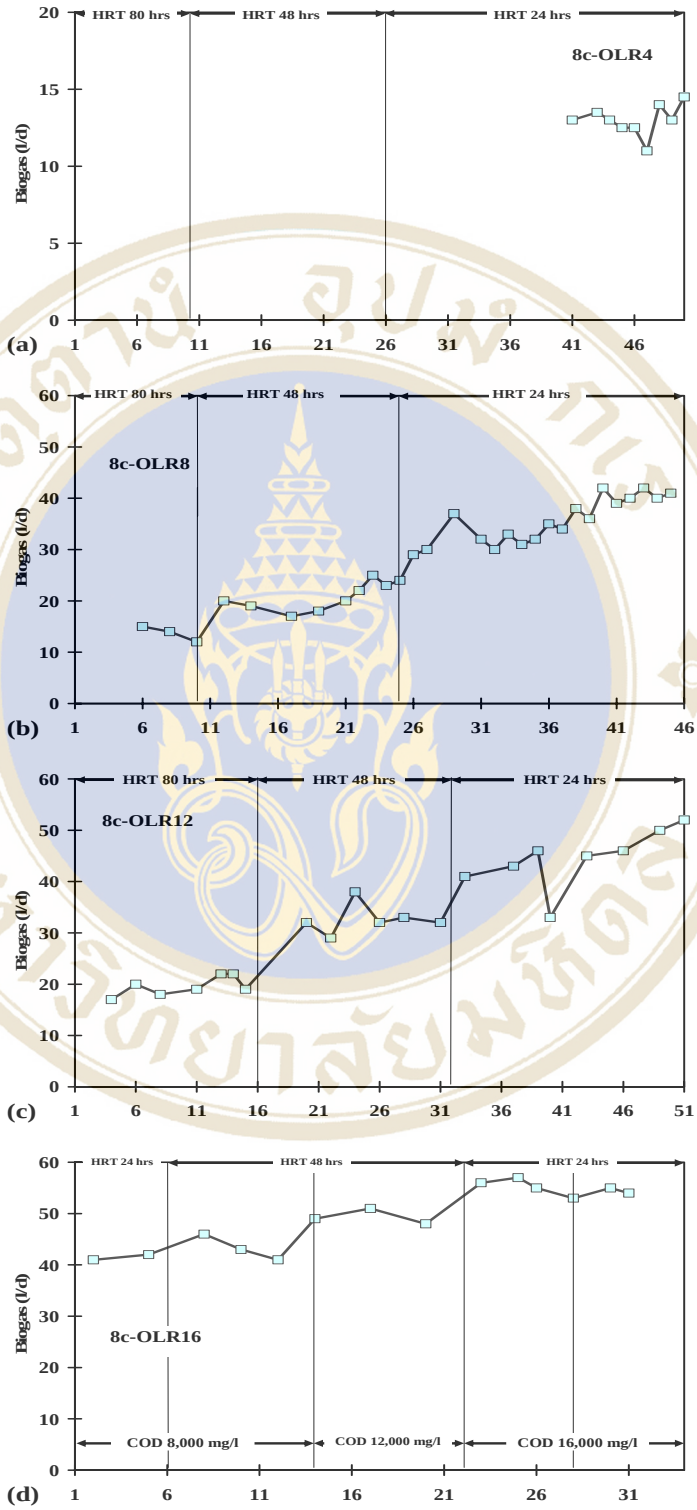


Figure 4.16 Biogas production rate of the experiments; (a) 8C-OLR4 (b) 8C-OLR48 (c) 8C-OLR12 (d) 8C-OLR16.

among the compartments. The ORP value was in range of -300 to -400 mV, as summarized in Table 4.8. However, ORP value in the second and/or the third compartments of every ABRs slightly decrease from the first compartment with unknown reason.

4.3.5 Biogas Production

Figure 4.16 showed biogas production in the experiment of 8C-OLR4, 8C-OLR8, 8C-OLR12, and 8C-OLR16 throughout the operation times. Average biogas production rates of the 8C-OLR4, 8C-OLR8, 8C-OLR12, and 8C-OLR16 experiment were 13, 40, 48, and 54 l/d, respectively. The results showed that biogas production increase every increased when OLR increase, even in the 8C-OLR16 experiment, which it steady-state could not be reached as the COD removal efficiency decreased to about 35%, biogas production rate before failure was still high.

4.3.6 Solid Retention Time

Effluent solids in this experimental part were shown in Figure 4.17. With the same eight compartments, the ability to retain solid within the reactor were pretty much the same in every ABRs. The effluent solid concentration was low during the early period of operation. When decreased HRT 24 hrs, a small peak of effluent solid concentration occurred, but then back to low concentration again when steady-state was reached. This confirms the assumption that the compartmentalized structure in ABRs could maintain high SRT while operated with low HRT. During the steady-state, effluent solids of the 8C-OLR4 and 8C-OLR8 experiments were less than 300 mg TSS/l, those of the 8C-OLR12 and 8C-OLR16 experiment were about 700 and 1,000 mg TSS/l, respectively. In case of the 8C-OLR16 experiment, the effluent solid at early operation was fluctuated and seeming high when compare with the other ABRs. There were about 1,000-1,500 mg TSS/l because this experiment was started with HRT of 24 hrs while the other ABRs were started up with HRT of 80 hrs.

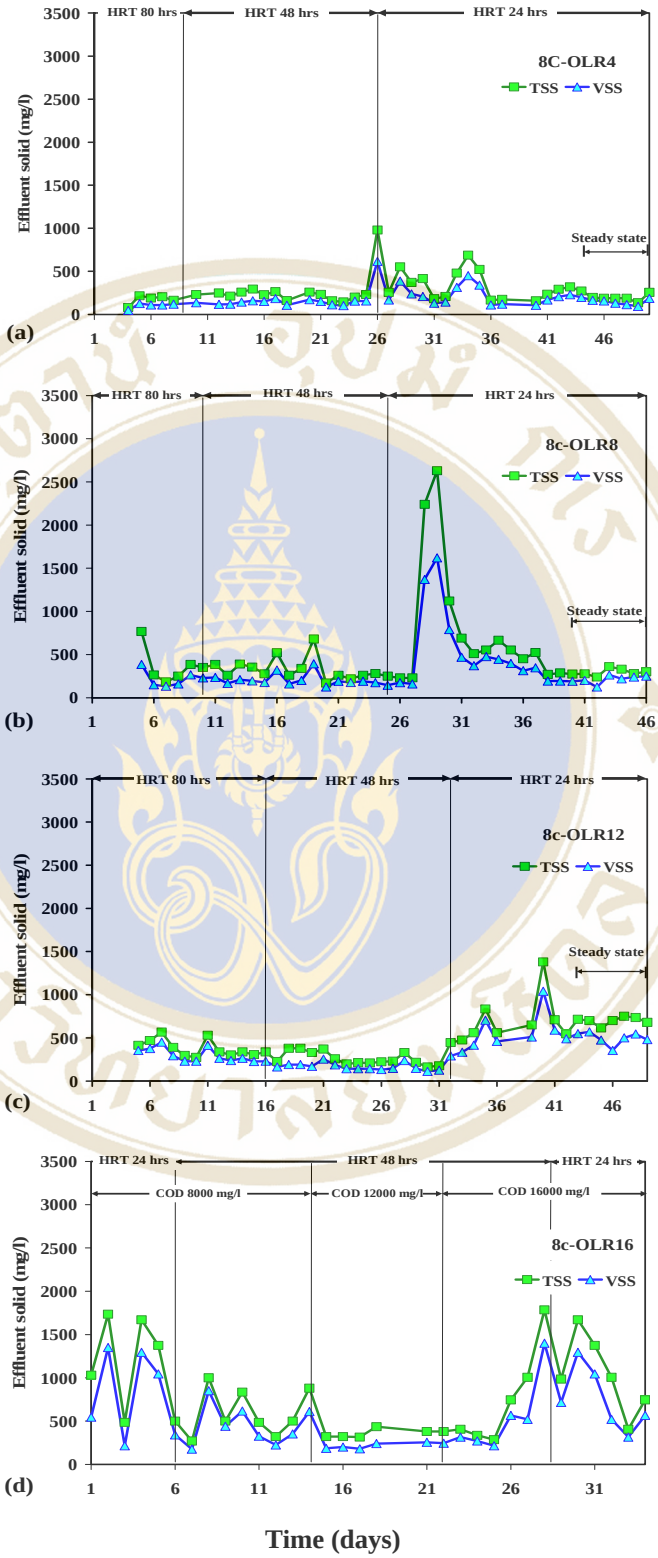


Figure 4.17 Overall effluent solid concentration of the experiments; (a) 8C-OLR4 (b) 8C-OLR48 (c) 8C-OLR12 (d) 8C-OLR16.

Table 4.9 The details of sludge in the experiments of Part I.

Parameters	Eq.	8C-OLR4		8C-OLR8		8C-OLR12		8C-OLR16	
		TSS	*VSS*	*TSS*	*VSS*	*TSS*	*VSS*	*TSS*	*VSS*
Initial sludge (g)	*(4.1)*	276	216	278	231	288	201	276	212
Final sludge (g)	*(4.2)*	257	193	256	232	321	218	378	165
Sludge washout rate (g)	*(4.3)*	1.9	1.4	2.9	2.1	7.0	4.9	10.3	7.4
Total sludge washout (g)	*(4.4)*	94	62	161	107	142	105	194	139
Sludge produce (g)	*(4.5)*	75	40	140	108	175	122	296	92
Yield (g VSS/ g COD)	*(4.6)*	0.03		0.04		0.05		0.01	
SRT (days)	***(1.1)***	**141**		**109**		**45**		**22**	

When each experimental finished, sludge in the reactor rinsed out and analyzed for calculation in Table 4.9. Results in Table 4.9 were calculated from equation 1.1 and 4.1-4.6.

From Table 4.9, seed sludge of every ABRs experiment in this part was about 28 g TSS/l. Final sludge and total sludge washout of the 8C-OLR4, 8C-OLR8, 8C-OLR12, and 8C-OLR16 experiment were 257, 256, 321, and 378 and 94, 161, 142, and 194 g TSS, respectively (Table 4.9 and Figure 4.18). Sludge washout rate of 8C-OLR4, 8C-OLR8, 8C-OLR12, and 8C-OLR16 were 1.9, 2.9, 7.0, and 10.3 g/d, respectively. Although ABRs used in this part were equipped with the same number of compartment, the SRT and SRT/HRT ratios were not similar. From this study, it can be seen that the OLR was affected on SRT and SRT/HRT ratio of ABR, higher OLR resulted in lower SRT and SRT/HRT ratio. The lower SRT and SRT/HRT ratio was involved with higher total sludge washout possibly due to more biogas production rate causing high turbulence in sludge bed. Normally, sludge in ABR was settled at the bottom of reactor and sometime raised and fell by wastewater flow and gas production. High OLR also could result in high biogas production, then, the sludge in ABR was more like a completely mix.

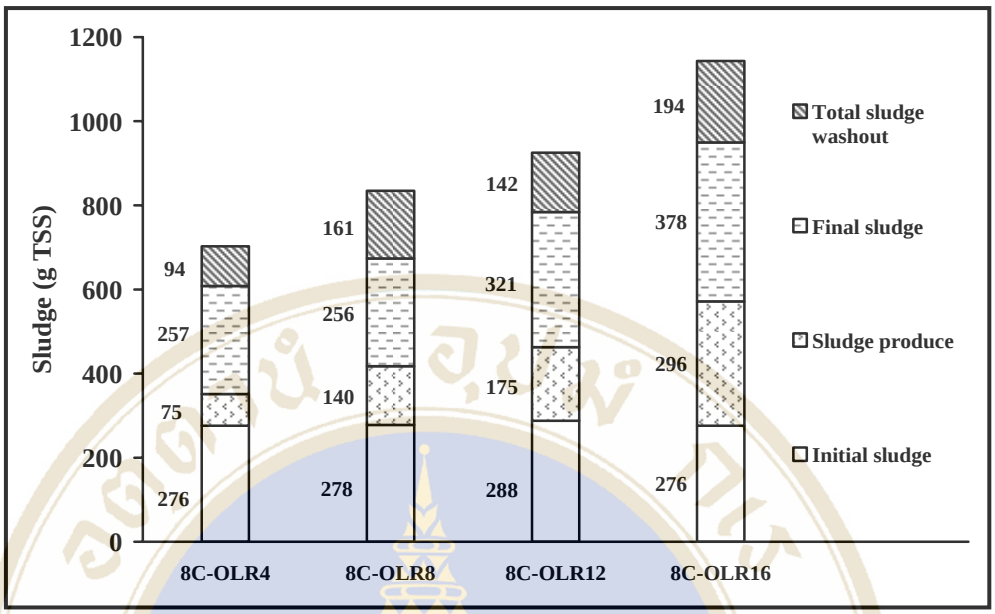


Figure 4.18 Sludge balance in the experiments of Part II.

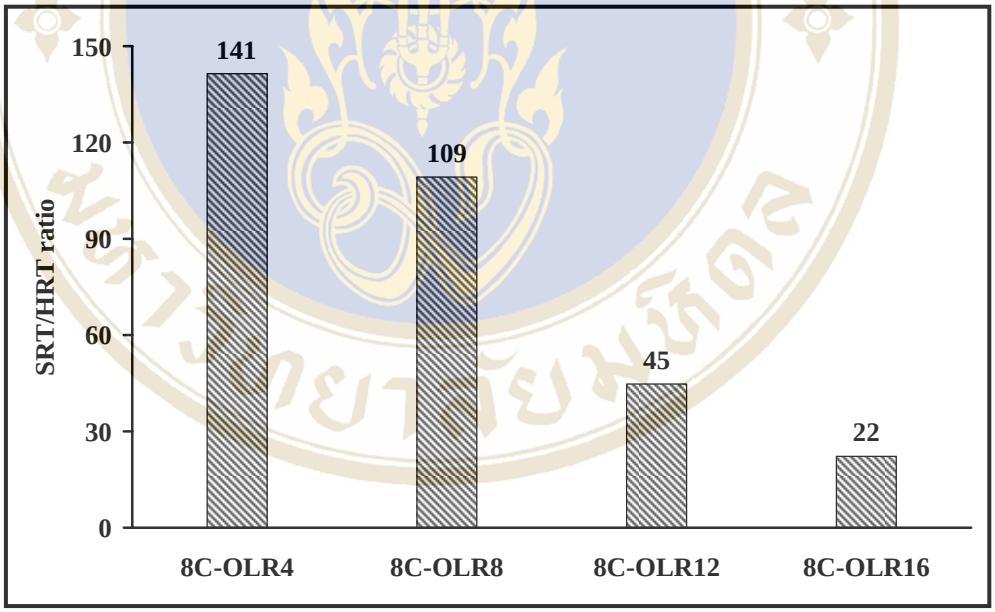


Figure 4.19 The SRT/HRT ratios in consideration of various OLRs.

The SRT/HRT ratios of the 8C-OLR4, 8C-OLR8, 8C-OLR12, and 8C-OLR16 experiment were 141, 109, 45, and 22 d/d, respectively, as shown in Figure 4.19. In addition, the OLR also affected on sludge yield which increased with increasing of OLR as shown in Table 4.9. However, sludge yield of the 8C-OLR16 was very low due to the system failure; the ratio of volatile fraction also support this mention (VSS/TSS=0.44). The ratio of volatile fraction final sludge of 8C-OLR8 experiment

was the highest (0.91), and those of the 8C-OLR4 and 8C-OLR12 experiment were 0.75 and 0.68, respectively.

4.3.8 Sub-conclusion

In biological wastewater treatment, an optimum OLR is a significant factor, because it affected the microbial ecology and performance characteristics of the system. Several reports mentioned about the applied OLR that depended on a number of factors, such as operational temperature, wastewater characteristics, and type of reactor and so forth. In view of that the objective of this study was to assess the applicable OLR for ABR.

To obtain OLR of 4, 8, 12, and 16 g COD/l-d, influent COD concentration were varied between 4,000-16,000 mg/l while hydraulic retention time was kept constant at 24 hrs HRT. The results obtained from the experiments showed that COD removal efficiency increased from 83 to 96% as the OLR increased from 4 to 8 g COD/l-d, and a little decreased to 88% as the OLR increases to 12 g COD/l-d. Eventually when the operating OLR was up to 16 g COD/l-d, the system could not maintain its stability and the failure occurred with the COD removal efficiency down to 34% as shown in Figure 4.20.

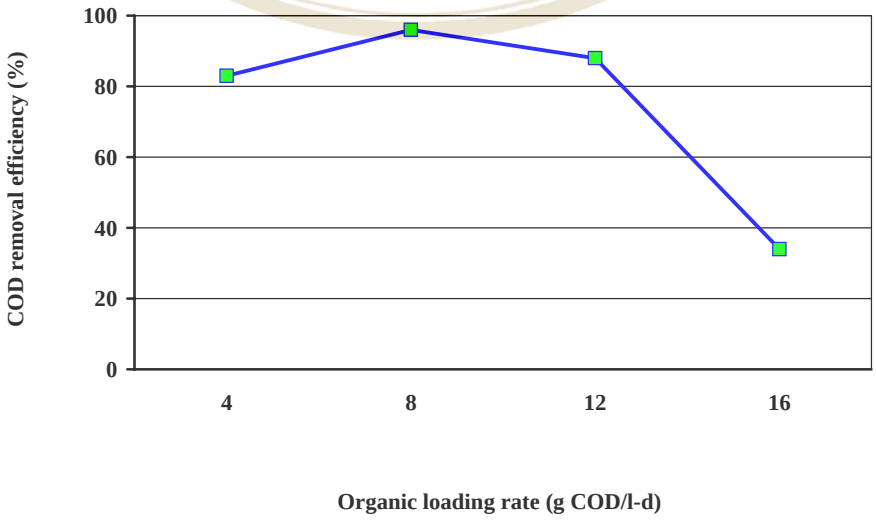


Figure 4.20 COD removal efficiencies of ABR with various organic loading rate.

Figure 4.20 indicates that the operating OLRs between 4 to 12 g COD/l-d could achieve the COD removal efficiency higher than 80%. However, the lower efficiency than 40% was occurred in the operation with 16 g COD/l-d. The failure of the experiment with 16 g COD/l-d OLR in this study could be caused by several reasons, not only high OLR, but also the rapid OLR raising.

As mentioned above, the optimum OLR applied for an ABR to obtain the high COD removal efficiency over 80% could be in range of 4 to 12 g COD/l-d, particularly in treating low solid content wastewater.

4.4 Fluorescence *in situ* Hybridization (FISH)

Several researchers observed that the configuration of ABR potentially enables for microbial phase separation could be occurred within the same reactor (Nachiyasit and Stuckey, 1995; Langenhoff and Stuckey, 2000; Langenhoff et al., 2000; Akunna and Clark, 2000). The front zone or near inlet acts like an acidogenic phase and the rear of the reactor performs a methanogenic phase. Acidogens and methanogens are the significant microorganism in the anaerobic processes, however, the wide distinction in term of physical, nutrition need, growth kinetics and sensitive environment parameters for both of them must be concerned. Pohland and Ghosh (1971) who are the first to described the physical separation of acidogens and methanogens. The optimum environmental conditions for both group of microorganisms should be provided to enhance the overall process stability and control (Demirel and Yenigun, 2002). Then, to authenticate this assumption, sludge in each compartment of ABRs were sampled at the end of each experiment for studying of microbial populations.

The microbial populations were studied by FISH technique (its procudure presented in appendix A) using probe EUB338 labeled with FITC (green) for domain *Eubacteria* which acidogens belong to this group of bacteria, and probe ARC915 labeled with CY3 (red) for domain *Archaea*, which methanogens were categorized into

this group. Also, DAPI (blue) staining was used to determine total cell bacteria. Due to this study would only justified the assumption of microbial phase separation in ABR system, then, only two groups of anaerobic microorganisms, i.e. acidogens and methanogens were considered. The FISH results were evident in identification of the prominent group of bacteria in each compartment hence determination of cell counts was not performed in this study.

Figure 4.21 shows FISH images of the 3C-OLR4 experiment. Among three compartments, the ratios of acidogens (EUB338 probed; green color) to total bacteria cell (DAPI stained; blue color) in each compartment were a lot like the ratios of methanogens (ARC915 probed; red color) to total bacteria cell (DAPI stained; blue color). This means that the populations of methanogens and acidogens were not distinguished between each compartment of the three-compartment ABR. This was consistent to the pH values that were 6.8, 6.9, and 7.1 in the first, second, and third compartments, respectively. It was obvious that a lesser amount of compartment was not promoting the microbial phase separation.

For the eight-compartment experiments, 8C-OLR8, 8C-OLR12, and 8C-OLR16, both acidogens and methanogens were clearly distinguished between each compartment. Acidogens (EUB338 probed; green) were a prominent group in the first compartments, and methanogens (ARC915 probed; red) were mostly prominent in the rear compartments as shown in Figures 4.22-4.24. The different OLRs have affected on total bacteria, which can be visually seen from the more dense DAPI staining images in the higher OLR.

Visually estimation of the ratios between acidogens and methanogens in each compartment of the 8C-OLR8 experiment was about 90:10 in the first compartment and approximately 70:30 in the third compartment. In the forth to the eighth compartments, acidogens were relatively lower than methanogens, the ratios of which were about 20:80 to 10:90 through the mentioned compartments (Figure 4.22).

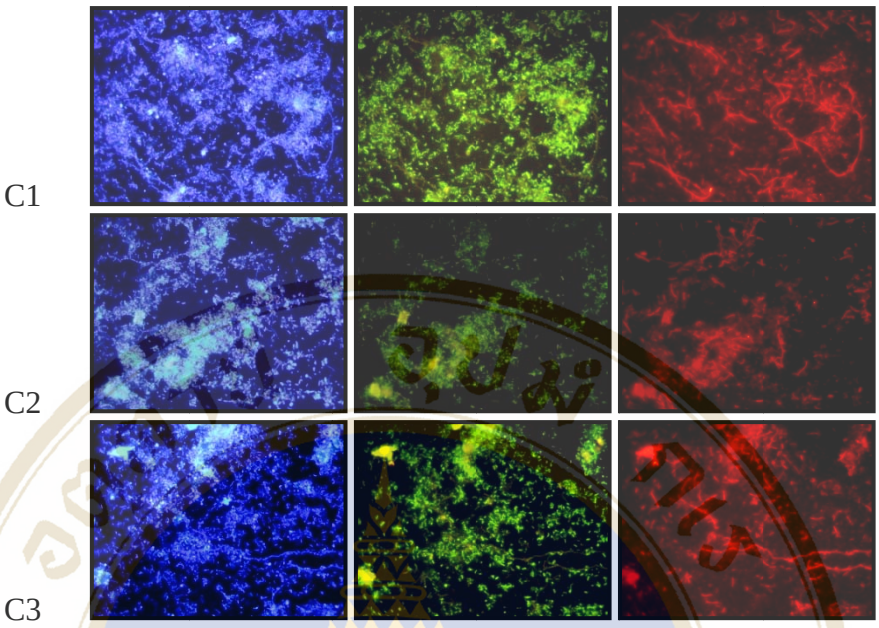


Figure 4.21 DAPI-staining and epifluorescence micrographs of microbial cells different compartment numbers at steady-state of **3C-OLR4** experiment; **Left**: DAPI staining, **Middle**: Bacterial cells hybridized with FITC-labeled EUB338 probes, and **Right**: Archaeal cells hybridized with Cy3-labeled ARC915 probe.

In the experiments of 8C-OLR12 and 8C-OLR16, the ratios of acidogens and methanogens in the first two compartments (pH values below 6.0) were about 90:10. Methanogens was prominent since the third compartments and the ratios of acidogens:methnogens were about 5:95 in the eighth compartments of both experiments (Figures 4.22 and 4.23). Even in the 8C-OLR16 experiment, which COD removal efficiency was continually decreased, a total microorganism within a reactor was still high (Figure 4.23). This may be because of two possible reasons, the first is FISH can not distinguish between alive with dead cells, and the second is VFA production rate of this OLR was exceeds the maximum consumption ability of methanogens, which resulted in VFA accumulation and pH dropped.

The ratios of acidogens and methanogens compared with total bacteria cell in the 8C-OLR8, 8C-OLR12, and 8C-OLR16 experiments were similar. Acidogens (EUB338 probed; green) was the prominent group in the front compartment and

methanogens (ARC915 probed; red) is in the rear compartment. **Therefore it can be concluded that acidogenic phase was evident in the first three compartments** where low pH values between of 5.4-6.7 occurred. **Methanogenic phase was manifested in the last four to five compartments** (pH values were about 7.0-7.8). In addition, the visual observation of sludge in different compartments found that sludge in the first three compartments was more whitish than those that were blackish in the latter compartments. This was similar to Cohen et al (1979) and others (Demirel and Yenigun, 2002) mention about sludge color was black in the methanogenic phase.

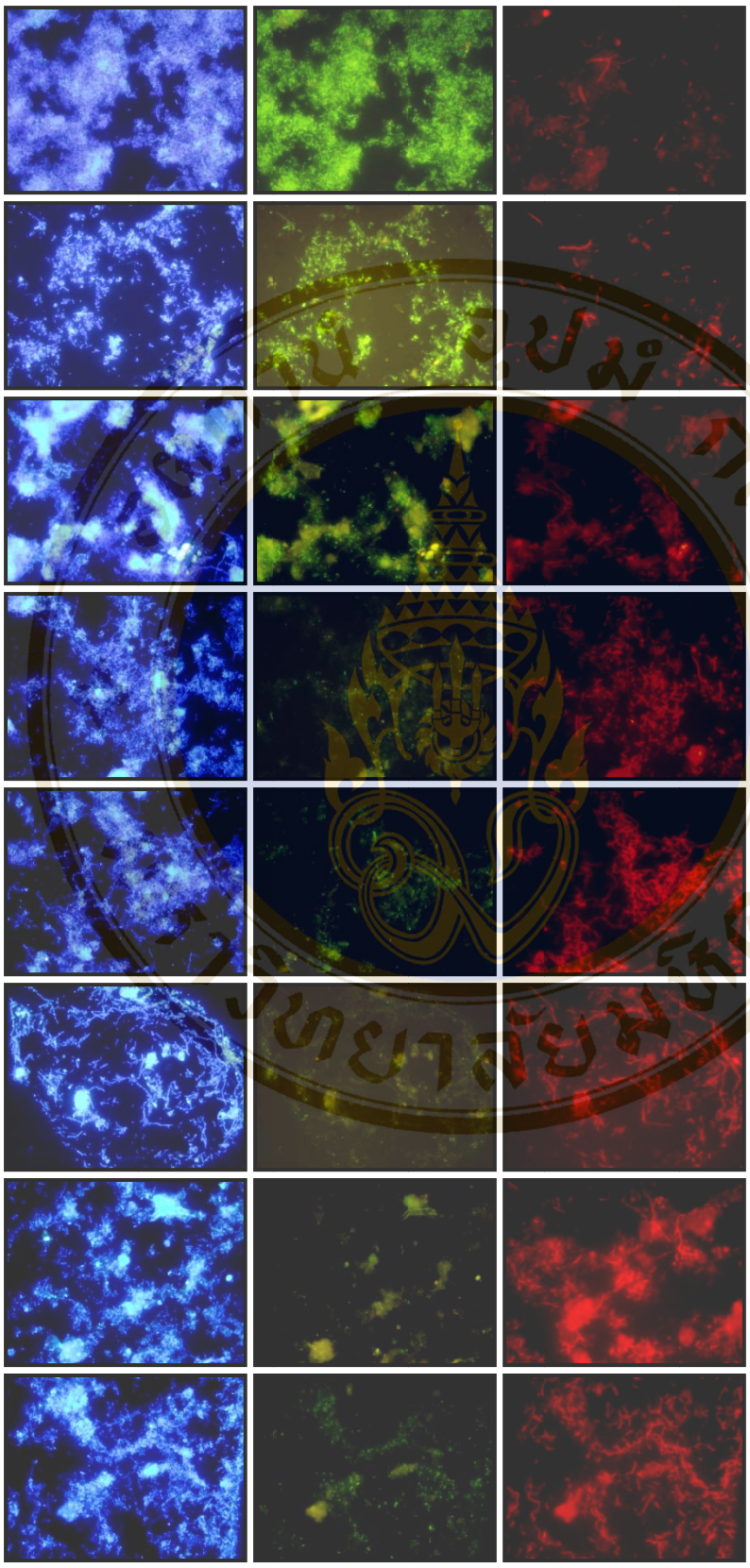


Figure 4.22 DAPI-staining and epifluorescence micrographs of microbial cells different compartment numbers at steady-state of **8C-OLR8** experiment; **Left**: DAPI staining, **Middle**: Bacterial cells hybridized with FITC-labeled EUB338 probes, and **Right**: Archaeal cells hybridized with Cy3-labeled ARC915 probe.

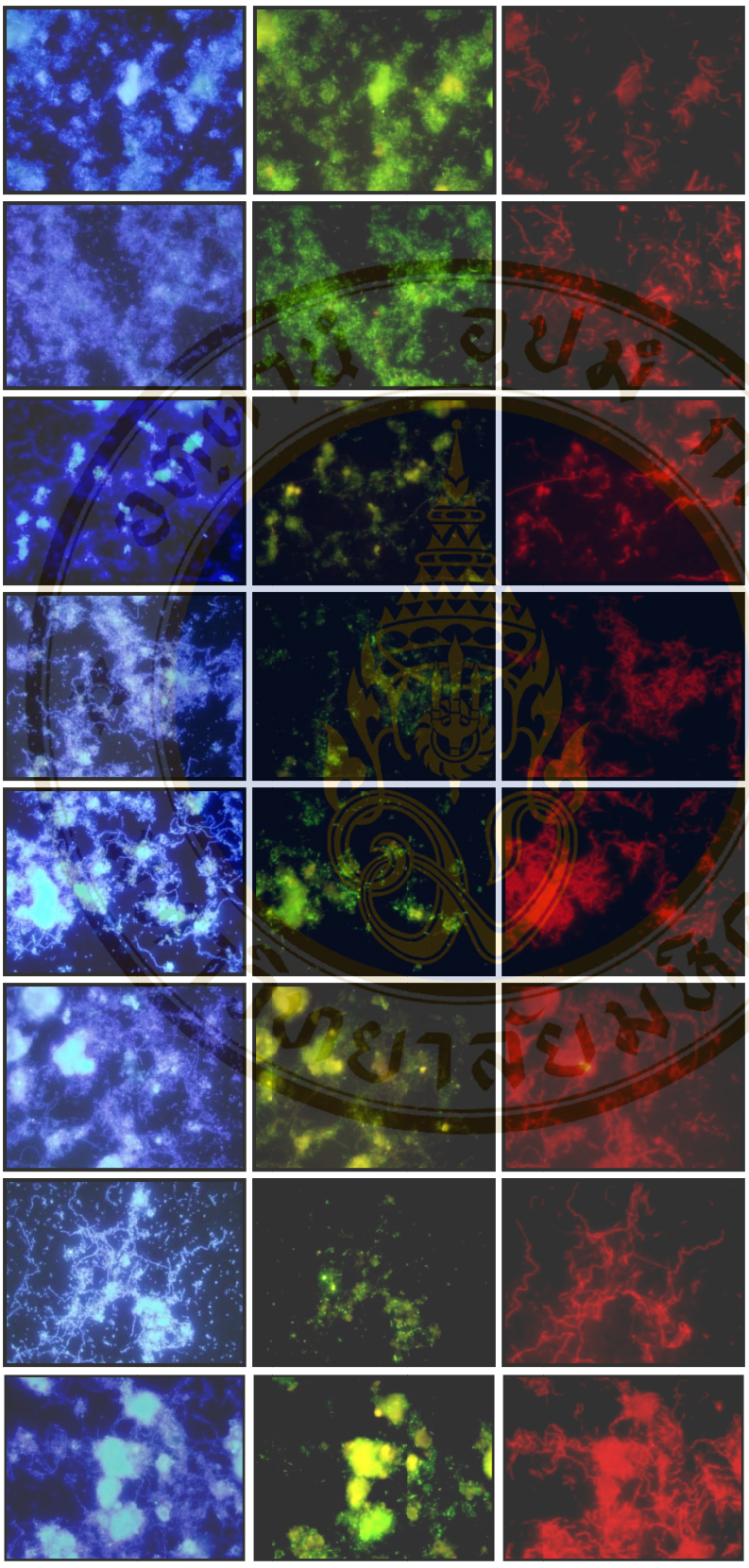


Figure 4.23 DAPI-staining and epifluorescence micrographs of microbial cells different compartment numbers at steady-state of **8C-OLR12** experiment; **Left**: DAPI staining, **Middle**: Bacterial cells hybridized with FITC-labeled EUB338 probes, and **Right**: Archaeal cells hybridized with Cy3-labeled ARC915 probe.

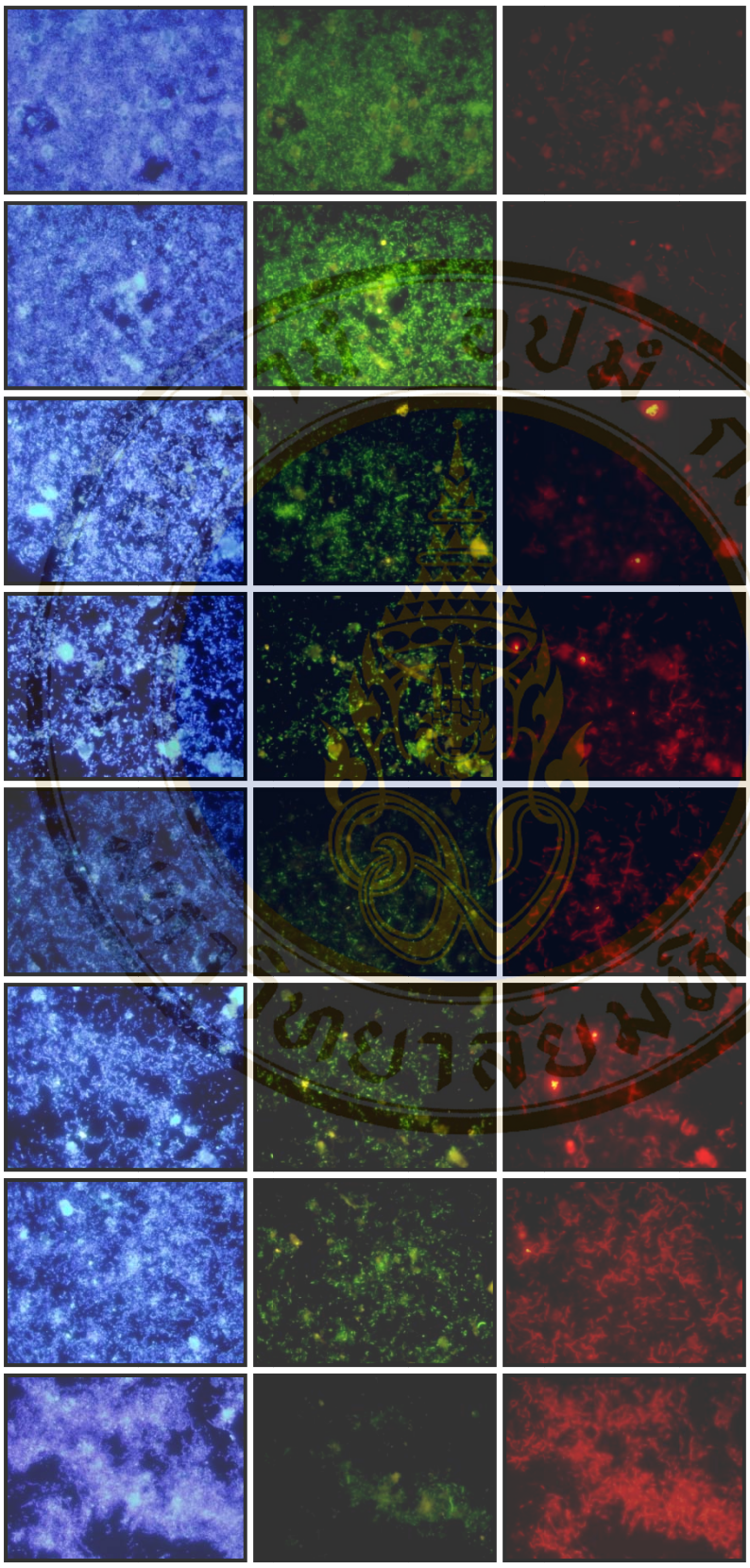


Figure 4.24 DAPI-staining and epifluorescence micrographs of microbial cells different compartment numbers at steady-state of **8C-OLR16** experiment; **Left**: DAPI staining, **Middle**: Bacterial cells hybridized with FITC-labeled EUB338 probes, and **Right**: Archaeal cells hybridized with Cy3-labeled ARC915 probe.

CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The aim of this study was to investigate the optimum SRT and SRT/HRT ratio and organic loading rate (OLR) of anaerobic baffled reactor (ABR) treating carbohydrate-protein wastewater. The compartmentalizing in this such a reactor is an important factor affecting on SRT and SRT/HRT ratio. More compartments helped retarding sludge washout, which could be confirmed by the results from this study. The COD removal efficiencies and SRT and SRT/HRT ratios increased with the numbers of compartment. The COD removal efficiencies of 74, 78, and 83% could be achieved in three-compartment, six-compartment, and eight-compartment ABRs, respectively. SRT obtained from those three reactors were 35, 74, and 134 day, while the SRT/HRT ratios were 35, 74, and 134 day/day, respectively. Therefore, the compartmentalized configuration was substantially resistant to sludge washout, resulting in higher SRT with smaller HRT and consequently better COD removal efficiency obtain from the eight-compartment ABR. Base on this study an optimum SRT/HRT ratio was 134 day/day. Although the microbial phase separation was not obvious in the three-compartment reactors, the COD removal efficiency was quite fair (over 70%) with the HRT of only 24 hrs.

The eight-compartment reactor was used to determine the optimum OLR in a range of 4 to 16 g COD/l-d. The COD removal efficiencies over 80% were achieved at operating OLRs between 4 to 12 g COD/l-d whereas the OLR of 16 g COD/l-d operation was not succeed. **Therefore, the eight-compartment ABR could be able to handle the operating ABR as high as 12 g COD/l-d with COD removal**

efficiency higher than 80%. However, this conclusion was recommended for the ABR treating a low solid content wastewater.

5.2 Recommendations

Interestingly, effluent pH and alkalinity values of all experiments were quite high though the influent alkalinity was controlled to be constant with the addition of 3,000 mg/l of sodium bicarbonate. This is possible to state that alkalinity requirement may be lessened than usual in ABR system, however, this mention needs to be further investigated.

From this study, recommended further investigation should be as the followings;

- Investigation the SRT/HRT ratio when treating with high solid content wastewater.
- Investigation of the possibility to reduce alkalinity addition in ABR system.
- Investigation the microbial communities varied with time series.
- Investigation of other factors affecting on the operating OLR such as operational temperature, wastewater characteristic and so on.

REFERENCES

Akunna, J.C. and Clark, M. (2000). Performance of a granular bed anaerobic baffled reactor (GRABBR) treating whisky distillery wastewater. *Bioresource and Technology*, 74, 257-264.

Alfreider, A., Pernthaier, J., Amann, R., Sattler, B., Glockner, F.O., Willw, A.and Psenner, R. (1996). Community analysis of the bacterial assemblages in the winter cover and pelagig layers of a high mountain lake by in situ hybridization. *Applied Environmental Microbiology*, 62, 2138-2144.

Anderson, K., Sallis, P. and Uyanik, S. (2003). Anaerobic treatment processes. In D. Mara, and N. Horan (Eds), *The handbook of water and wastewater microbiology* (pp. 391-426). London: Academic Press.

Angelidakia, I., Chena, X., Cuia, J., Kaparajua, P. and Ellegaardb, L. (2006). Thermophilic anaerobic digestion of source-sorted organic fraction of household municipal solid waste Start-up procedure for continuously stirred tank reactor. *Water Research*, 40, 2621 – 2628.

APHA., AWWA., WPC. (1998). *Standard methods for the examination of water and wastewater (20th ed.)*. Washington DC, American Public Health Association, American Water Work Association, Water Pollution Control.

Azbar, N., Ursillo, P. and Speece, R.E. (2001). Effect of process configuration and substrate complexity on the performance of anaerobic processes. *Water Research*, 35(3), 817-829.

Bachmann, A., Beard, V.L. and McCarty, P.L. (1985). Performance characteristics of the anaerobic baffled reactor. *Water Research*, 19(1), 99-106.

Baloch, M.I., Akunna, J.C. and Collier, P.J. (2007). The performance of a phase separated granular bed bioreactor treating brewery wastewater. *Bioresource Technology*, 98, 1849-1855.

Barber, W.P. and Stuckey, D.C. (1998). The influence of start-up strategies on the performance of an anaerobic baffled reactor. *Environmental Technology*, 19, 489-501.

Barber, W.P. and Stuckey, D.C. (1999). The use of the anaerobic baffled reactor (ABR) for wastewater treatment: A review. *Water Research*, 33(7), 1559-1578.

Barber, W.P. and Stuckey, D.C. (2000a). Nitrogen removal in a modified anaerobic baffled reactor (ABR): 1 Denitrification. *Water research*, 34(9), 2413-2422.

Barber, W.P. and Stuckey, D.C. (2000b) Nitrogen removal in a modified anaerobic baffled reactor (ABR): 2 Nitrification. *Water research*, 34(9), 2423-2432.

Behling, E. et al. (1997). Domestic wastewater treatment using a UASB reactor. *Bioresource Technology*, 61, 239-245.

Bell, J. and Buckley, C.A. (2003). Treatment of a textile dye in the anaerobic baffled reactor. *Water SA*, 29(2), 129-134.

Bell, J., Plumb, J.J., Buckley, C.A. and Stuckey, D.C. (2000). Treatment and decolorization of dyes in an anaerobic baffled reactor. *Journal of Environmental Engineering*, 1026-1032.

Bitton, G. (2005). *Wastewater microbiology (3rd ed.)*. New Jersey: John Wiley and Sons, Inc.

Boonapatcharoen, N. Characterization of microbial community in granular sludge treating cassava starch wastewater by 16S rRNA approach. [M.S. Thesis in Biotechnology] Bangkok; School of Bioresources and Technology, King Mongkut's University of Technology Thonburi; 2003.

Boopathy, R. (1998). Biological treatment of swine waste using anaerobic baffled reactors. *Bioresource Technology*, 64, 1-6.

Boopathy, R. and Sievers, D.M. (1991). Performance of a modified anaerobic baffled reactor to treat swine waste. *Transactions of the ASAE*, 34(6), 2573-2578.

Boopathy, R. and Tilche, A. (1991). Anaerobic digestion of high strength molasses wastewater using hybrid anaerobic baffled reactor. *Water Research*, 25(7), 785-790.

Boopathy, R. and Tilche, A. (1992). Pelletization of biomass in Hybrid Anaerobic Baffled Reactor (HABR) treating acidified wastewater. *Bioresource Technology*, 40, 101-107.

Boopathy, R., Larsen, V.F. and Seniou, E. (1988). Performance of Anaerobic Baffled Reactor (ABR) in treating distillery waste water from a Scotch whisky factory. *Biomass*, 16, 133-143.

Dama, P. et al. (2001). Flow patterns in an anaerobic baffled reactor. Proceedings of the 9th World Congress on Anaerobic Digestion, Antwerpan, Belgium, 2-5 September, 798. 793.

Dama, P. et al. (2002). Pilot-scale study of an anaerobic baffled reactor for the treatment of domestic wastewater. *Water Science and Technology*, 46(9), 263-270.

Demirer, G.N. and Chen, S. (2005). Two-phase anaerobic digestion of unscreened dairy manure. *Process Biochemistry*, 40(11), 3542-3549

Fannin, K.F. (1987). Start-up, operation, stability, and control. In D.P. Chynoweth, and R. Isaacson. (Eds.), *Anaerobic digestion of biomass* (pp. 171-193). Great Britain: University Press, Cambridge.

Foxon, K.M., Pillay, S., Lalbahadur, T., Rodda, N., Holder, F. and Buckley, C.A. (2004). The anaerobic Baffled Reactor (ABR), An appropriate technology for on-site sanitation. *Water SA 30(5)*, special edition, 44-50.

Freese, L.H. and Stuckey, D.C. (2000). Influence of seed inoculum on the start-up of an anaerobic baffled reactor. *Environmental Technology*, 21, 909-918.

Grady, C.P., Daigger, G.T. and Lim, H.C. (1999). *Biological wastewater treatment (2nd ed.).* USA: Marcel Dekker,Inc.

Grobicki, A. and Stuckey, D.C. (1991). Performance of the anaerobic baffled reactor under steady-state and shock loading condition. *Biotechnology and Bioengineering*, 37, 344-355.

Grobicki, A. and Stuckey, D.C. (1992). Hydrodynamic characteristics of the anaerobic baffled reactor. *Water Research*, 26(3), 371-378.

Grover, R., Marwaha, S.S. and Denedy, J.F. (1999). Studies on the use of an anaerobic baffled reactor for the continuous anaerobic digestion of pulp and paper mill black liquors. *Process Biochemistry*, 34, 653-657.

Gujer, W. and Zehnder, A.J.B. (1983). Conversion processes in anaerobic digestion. *Water Science and Technology,* 15, 127-167.

Juparaputtasri, W., Boonapatchareon, N., Cheevadhanarak, S., Chaiprasert, P., Tanticharoen, M. and Techkarnjanaruk, S. (2005). Use of an alternative Archaea-specific probe for methanogen detection. *Journal of Microbiological Methods,* 61, 95-104.

Kalmbach, S., Manz, W., Wecke, J. and Szewzyk, U. (1999). Aquabacteriumgen. nov., with description of *Aquabacterium citratiphilum* sp. nov., *Aquabacterium parvum* sp. nov. and *Aquabacterium commune* sp. nov., three in situ dominate bacterial species from the Berlin drinking water system. *International Journal of Systematic Bacteriology,* 49, 769-777.

Kim, D.J., Kim, T.K., Choi, E.J., Kim, T.H., Ahn, D.H., Yuan, Z., et al., (2004). Fluorescence in situ hybridization analysis of nitrifiers in piggery wastewater treatment reactors. *Water Science and Technology,* 49(5-6), 333-340.

Kuscu, O.S. and Sponza, D.T. (2006). Treatment efficiencies of a sequential anaerobic baffled reactor (ABR)-completely stirred tank reactor (CSTR) system at increasing p-nitrophenol and COD loading rates. *Process biochemistry,* 41, 1484-1492.

Langenhoff, A.A.M. and Stuckey, D.C. (2000). Treatment of dilute wastewater using an anaerobic baffled reactor: Effect of low temperature. *Water Research, 34(15),* 3867-3875.

Langenhoff, A.A.M., Intrachandra, N. and Stuckey, D.C. (2000). Treatment of dilute soluble and colloidal wastewater using an anaerobic baffled reactor, Influence of hydraulic retention time. *Water research, 34(4),* 1307-1317.

Liu, W.T., Chan, C.O. and Fang, H.H.P. (2002). Microbial community dynamics during start-up of acidogenic anaerobic reactor. *Water Research,* 36, 3203-3210.

Llobet-Brossa, E., Rossello-Mora, R. and Amann, R., (1998). Microbial community composition of Wadden Sea sediments as revealed by fluorescence community composition of Wadden Sea sediments as revealed by

fluorescence in situ hybridization. *Applied and Environmental Microbiology,* 64, 2691-2696.

MacNaughton, S.J., Booth, T., Embley, T.M. and O'Donnell, A.G. (1996). Physical stabilization and confocal microscopy of bacteria on roots using 16S rRNA targeted fluorescent-labeled oligonucleotide probes. *Journal of Microbiological Methods,* 26, 279-285.

Metcalf and Eddy (2004). *Wastewater engineering treatment and reuse (4th ed.).* Singapore: Mc Graw Hill.

Moter, A. and Göbel, U.B. (2000). Fluorescence in situ hybridization (FISH) for direct visualization of microorganisms. *Journal of Microbiological Methods, 41(2),* 85-112.

Nachaiyasit, S. and Stuckey, D.C. (1997a). Effect of low temperatures on the performance of an anaerobic baffled reactor (ABR). *Journal of Chemical Technology and Biotechnology,* 69, 276-284.

Nachaiyasit, S. and Stuckey, D.C. (1997b). The effect of shock loads on the performance of an anaerobic baffled reactor (ABR): 1, step changes in feed concentration at constant retention time. *Water Research,* 31(11), 2737-2746.

Nachaiyasit, S. and Stuckey, D.C. (1997c). The effect of shock loads on the performance of an anaerobic baffled reactor (ABR): 2, step and transient hydraulic shocks at constant feed strength. *Water Research,* 31(11), 2747-2754.

Nachiyasit, S. and Stuckey, D. (1995). Microbial response to environmental changes in an anaerobic baffled reactor (ABR). *Antonie van Leeuwenhoek,* 67, 111-123.

Plumb, J.J., Bell, J. and Stuckey, D.C. (2001). Microbial populations associated with treatment of an industrial dye effluent in an anaerobic baffled reactor. *Apply and Environmental Microbiology,* 67(7), 3226-3235.

Polprasert, C., Kemmadamrong, P. and Tran R.T. (1992). Anaerobic Baffled Reactor (ABR) process for treating a slaughterhouse wastewater. *Environmental Technology,* 13, 857-865.

Reynoles, T.D. and Richards, P.A. (1996). *Unit operations and processes in environmental engineering (2nd ed.).* Boston: PWS Publishing Company.

Schramm, A., Santegoeds, C.M., Nielsen, H.K., Ploug, H., Wagner, M., Pribyl, M., et al., (1999). On the occurrence of anoxic microniches, denitrification, and sulfate reduction in aerated activated sludge,"microniches, denitrification, and sulfate reduction in aerated activated sludge. *Applied and Environmental Microbiology,* 65, 4189-4196.

Sekiguchi, Y., Kamagata, Y., Nakamura, K., Ohashi, A., and Harada, H. (199). Fluorescence In Situ Hybridization Using 16S rRNA-Targeted Oligonucleotides Reveals Localization of Methanogens and Selected Uncultured Bacteria in Mesophilic and Thermophilic Sludge Granules. *Applied Environmental Microbiology,* 65(3), 1280–1288.

She, Z., Zheng, X., Yang, B., Jin, C. and Gao, M. (2006). Granule development and performance in sucrose fed anaerobic baffled reactors. *Journal of Biotechnology,* 122, 198-208.

Singh, K.S. and Viraraghavan, T. (2003). Impact of temperature on performance, microbiological, and hydrodynamic aspects of UASB reactor treating municipal wastewater. *Water Science and Technology*, 48(6), 211-217.

Singh, R.P., Kumar, S. and Ojha, C.S.P. (1999). Nutrient requirement for UASB process: a review. *Biochemical Engineering Journal,* 3, 35-54.

Skiadas, I.V. and Lyberatos, G. (1998). The periodic anaerobic baffled reactor. *Water Science and Technology*, 38(8-9), 401-408.

Torkian, A., Eqbali, A. and Hashemian, S.J. (2003). The effect of organic loading rate on the performance of UASB reactor treating slaughterhouse effluent. Resources, Conservation and Recycling, *(Inpress).*

Udomsinrot, K. (2000). *Wastewater engineering Vol.4.* Patumthani: Thailand. (In Thai)

Uyanik, S., Sallis, P.J. and Anderson, G.K. (2002). The effect of polymer addition on granulation in an anaerobic baffled reactor (ABR): Part I, Process performance. *Water Research,* 36, 933-943.

Vossoughi, M., Shakri, M. and Alemzadeh, I. (2003). Performance of anaerobic baffled reactor treating synthetic wastewater influences by decreasing COD/SO4 ratios. *Chemical Engineering and Processing*, 42, 811-816.

Wagner, M., Nielsen, P.H., Loy, A., Nielsen, J.L. and Daims, H. (2006). Linking microbial community structure with function: fluorescence in situ hybridization-microautoradiography and isotope arrays. *Current Opinion in Biotechnology*, 17(1), 83-91.

Wu, H, Wang, S., Kong, H., Liu, T. and Xia, M. 2007. Performance of combined process of anoxic baffled reactor-biological contact oxidation treating printing and dyeing wastewater. *Bioresource Technology*, 98, 1501-1504.

Yacob, S., Shirai, Y., Hassan, M.A., Wasidaka, M. and Subash, S. (2006). Start-up operation of semi-commercial closed anaerobic digester for palm oil mill effluent treatment. *Process Biochemistry*, 41, 962-946.

APPENDIX

APPENDIX A

Fluorescence *in situ* hybridization (FISH) procedure

This procedure was adapted from Boonapatcharoen (2003).

1. Chemicals and Solutions

1) Fixative reagent (4% paraformaldehyde)

- Fixative reagent was used for fixation of bacteria cells to preserve ribosomal RNA (rRNA). Fixative reagent was prepared by adding 2 g paraformaldehyde slowly into 33 ml of dH_2O (add one drop of 10 M NaOH into dH_2O). The mixture was heated gently to 60°C and stirred until dissolved (do not over heat). 3x PBS (16.5 ml) was added into paraformaldehyde solution and filtered sterile through a disposable filter. The fixative reagent was kept on ice and used within 24 hours.

2) Acid alcohol

- Acid alcohol was used for cleaning slides before poly-L-lysine coating. Acid alcohol was prepared by adding 1% HCl (1ml) into 70% ethanol (99 ml).

3) 3x PBS bufffer

- 3x PBS buffer (390 mM of NaCl in 30 mM of $NaPO_4$ buffer) was prepare by adding 23.4 ml of 5 M NaCl to 18.0 ml of 0.5 M NaP $NaPO_4$ buffer. The pH was adjusted to 7.2 and final volume was adjusted with dH_2O to 300 ml. (0.5 M $NaPO_4$ buffer was prepared by mixing 28 ml of 0.5 M NaH_2PO_4 with 0.5 M Na_2HPO_4.)

4) Hybridization buffer

- Hybridization buffer contained 0.9 M NaCl, 0.01% SDS, 20 mM Tris-HCl (pH 7.2), and Vol% formamide (depending on probe, list in Table A-2).

5) Washing buffer

- The washing buffer contained X M NaCl, 0.01% SDS, and 20 mM Tris-HCl (pH 7.2). The concentration of NaCl in washing buffer are given at different concentrations of formamide in hybridization buffer (Table A-1).

6) DAPI solution

- DAPI was used for counterstaining after whole cell hybridization. Stock solution was prepared by adding dH_2O or dimethylformamide (DFM) to 100 µM. The stock solution was stored at 4°C. For counterstaining protocol, the DAPI stock solution was diluted to 300 nM in PBS. The working solution (300 nM) was added on the fixed cells and incubated at room temperature for 5 min. After that, slides were rinsed by dH_2O.

Table A-1 Preparation of washing buffer (50 ml).

% FA	NaCl (M)	X ml of 3M NaCl	% FA	NaCl (M)	X ml of 3M NaCl
0	0.900	15.0	45	0.040	0.67
5	0.636	10.6	50	0.028	0.47
10	0.450	7.5	55	0.020	0.33
15	0.318	5.30	60	0/014	0.23
20	0.225	3.75	65	0.010	0.17
25	0.159	2.65	70	0.007	0.12
30	0.112	1.86	75	0.005	0.08
35	0.080	1.33	80	0.0035	0.06
40	0.056	0.93			

2. Cell fixation and dispersion of sample

1) The sludge samples were dispersed by homogenizer, and then fixed in freshly prepared 4% paraformaldehyde solution at 4°C for 2-24 h immediately after the samples were taken.

2) Fixed samples were subsequently washed twice in 1X PBS (pH 7.2) and stored in a 1:1 mixture of 1X PBS and 96% ethanol at –20°C. Before application to filters or slides, fixed samples were diluted with 1X PBS (pH 7.2) and sonicated for 30-60 second at 2 W with a probe (diameter 2 mm).

3. Slide preparation

1) Slides were cleaned in acid alcohol (1-2% HCL in 70% ethanol) for 5 min and placed slides into 0.01% poly-L-lysin at room temperature for 5 min.

2) The coated slides were air dried for 1 hour at 60°C or overnight at room temperature and ready to use.

Table A-2 Sequence of 16S rRNA oligonucleotide probes used in this study.

Probe names	Specificity	Oligonucleotide sequence (5′- 3′)	rRNA Targets	% Formamide	Reference
EUB338	Domain Bacteria	GCTGCCTCCCGTA GGAGT	338-355	15	Stahl and Amann,1991
ARC915	Domain *Archaea*	GTGCTCCCCCGCCA ATTCCT	915-934	15	Amann et al., 1990

4. *In situ* hybridization

1) Fixed samples cells were spotted on coated slides (3-5 µl/sample) and air dried at 37°C or room temperature for 10-15 min.

2) Dried slides were dehydrated with ethanol series (50, 80 and 99%; 3 min/each), then air dried at room temperature.

3) After that, samples were hybridized with probes (Table 3) at 46°C for 60-90 min with 9 µl of hybridization buffer and 1 µl of probes (50 ng/µl). Hybridization stringency was adjusted by adding formamide to hybridization buffer (15-35%).

4) After hybridization, the slides were washed at 48°C for 15 min in washing buffer. Washing buffer was removed with distilled water, and then the slides were air-dried and mounted with anti-fading (supplied by Molecular Probes).

5) The slides were viewed on an epifluorescence microscope as described below.

5. Microscopy

Cells were examined with an epifluorescence microscope (Olympus model BX60, Japan) equipped with the following filter sets: MWU for DAPI (excitation 330-385 nm) and MWG for Cy3 (excitation 510-550 nm). Images were acquired using a charge-couple device (CCD) camera (Olympus model DP50, Japan). Exposure times were 0.04 to 0.06 s for phase contrast and 15-30 s for epifluorescence micrographs.

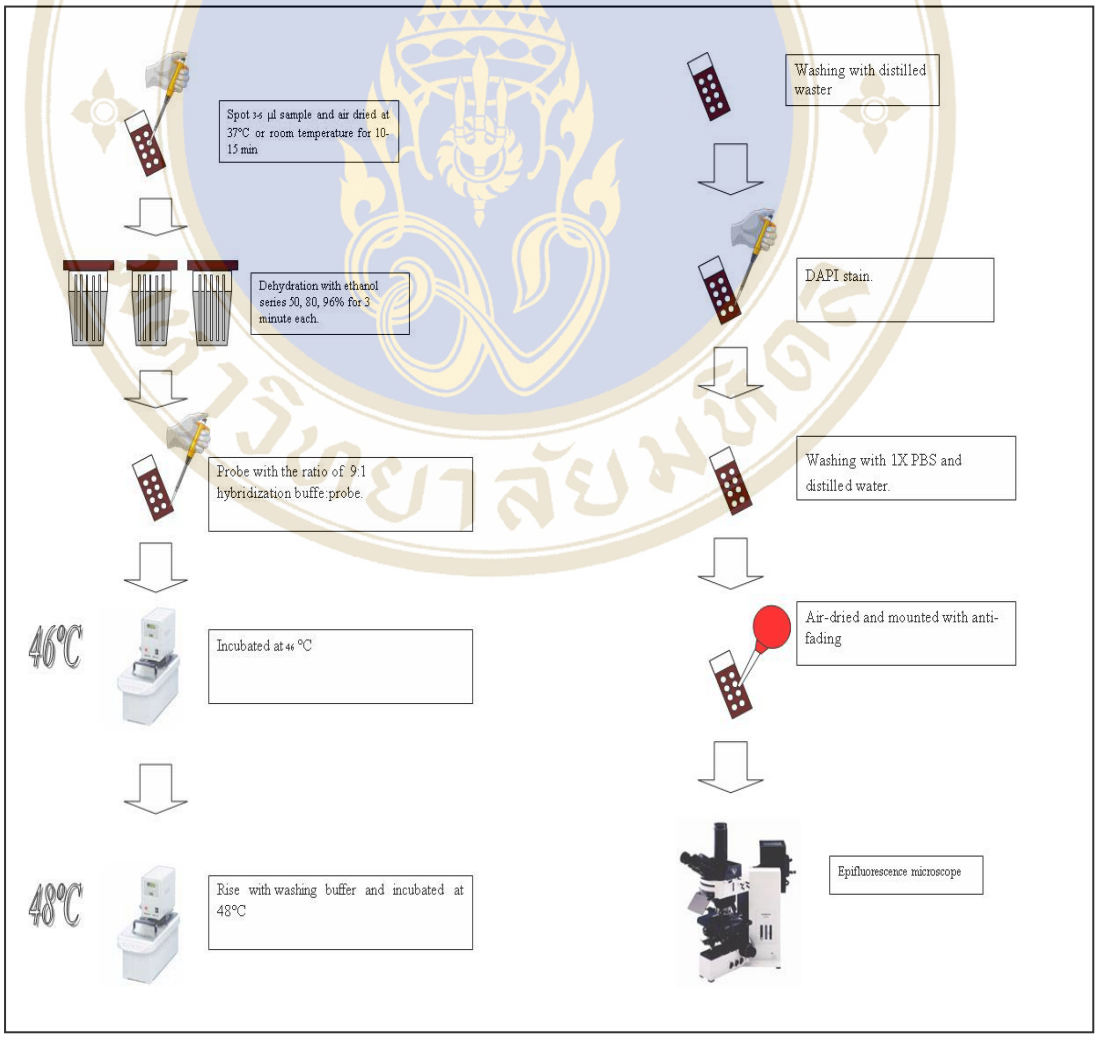


Figure A-1 FISH procedure.

BIOGRAPHY

NAME	Apaporn Ruchirased
DATE OF BIRTH	May 27, 1981
PLACE OF BIRTH	Singburi, Thailand
INSTITUTIONS ATTENDED	Burapha University (2000-2003) Bachelor of Science (Environmental Health) Mahidol University (2003-2006) Master of Science (Environmental Technology)
HOME ADDRESS	153 M. 1, T. Progruam, A. Muang, Singburi Thailand 16000 Tel. 0-3652-0459.