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TREATMENT OF EMBALMING FLUID USING DOMESTIC WASTEWATER AS CO-SUBSTRATE IN ANAEROBIC FILTER.

Miss Wanawan Pragot

A Thesis Submitted in Partial Fulfillment of the Requirements
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By	Miss Wanawan pragot
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Thesis Advisor	Patiroop Pholchan, Ph.D.

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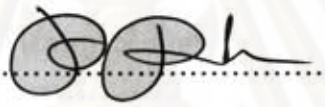

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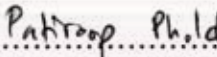

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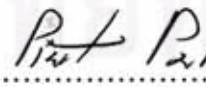

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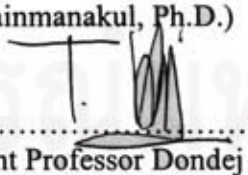

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วนาวัลย์ ปรากฏ: การบำบัดน้ำยาดองศพโดยใช้น้ำเสียชุมชนเป็นสารอาหารร่วมในถังกรองไร้ออกซิเจน (TREATMENT OF EMBALMING FLUID USING DOMESTIC WASTEWATER AS CO-SUBSTRATE IN ANAEROBIC FILTER.) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : ดร. ปฏิรูป ผลจันทร์, 127 หน้า.

การย่อยสลายทางชีวภาพของฟอร์มัลดีไฮด์และฟีนอลซึ่งเป็นองค์ประกอบหลักของน้ำยาดองศพโดยใช้น้ำเสียชุมชนเป็นสารอาหารร่วมในถังกรองไร้ออกซิเจนถูกศึกษาในงานวิจัยนี้ โดยทำการดำเนินการทดลองในถังกรองไร้ออกซิเจน 2 ถัง ที่ค่าเวลากักเก็บเท่ากับ 6 และ 12 ชั่วโมง เป็นระยะเวลา 215 วัน ส่วนผสมของน้ำยาดองศพและน้ำเสียชุมชนมีความเข้มข้นของฟอร์มัลดีไฮด์และฟีนอลเท่ากับ 22-1373 และ 5.4 -208 มก/ล ซึ่งเทียบเท่ากับความเข้มข้นซีโอดี 207-1756 มก./ล. ถูกป้อนเข้าสู่ทั้งสองถังปฏิกรณ์ ประสิทธิภาพการกำจัดฟอร์มัลดีไฮด์ที่ได้ในถังปฏิกรณ์ที่เวลากักเก็บ 6 และ 12 ชั่วโมงมีค่าเท่ากับ 97.0% และ 97.2% ทุกค่าความเข้มข้นของฟอร์มัลดีไฮด์ที่ใช้ในทางกลับกัน ฟีนอลถูกกำจัดได้อย่างสมบูรณ์ เมื่อความเข้มข้นเริ่มต้นมีค่าไม่เกิน 5.4 และ 33 มก./ล. ที่ความเข้มข้นของฟอร์มัลดีไฮด์เท่ากับ 64 และ 128 มก./ล. ในถังปฏิกรณ์ที่มีเวลากักเก็บที่ 6 และ 12 ชั่วโมงตามลำดับ เป็นไปได้ว่า ความเข้มข้นที่สูงของฟอร์มัลดีไฮด์มีผลเสียต่อจุลชีพบางชนิดที่ทำหน้าที่ย่อยสลายฟีนอล ผลที่ได้ยังแสดงให้เห็นว่าถังกรองไร้ออกซิเจนที่ดำเนินระบบที่ค่าเวลากักเก็บนานกว่า (12 ชั่วโมง) มีประสิทธิภาพในการกำจัดสารพิษที่ศึกษาสูงกว่าที่เวลากักเก็บสั้น (6 ชั่วโมง) เป็นไปได้ว่า ความเป็นพิษอย่างเฉียบพลันของฟอร์มัลดีไฮด์และฟีนอลส่งผลต่อการลดลงของกิจกรรมของจุลชีพ ยิ่งไปกว่านั้น น้ำยาดองศพในน้ำเข้าระบบยังทำให้เกิดการลดลงของค่ามีเทนจำเพาะ อัตราส่วนของน้ำยาดองศพต่อน้ำเสียชุมชนสูงสุดที่สามารถบำบัดได้ที่พบในงานวิจัยนี้มีค่าเท่ากับ 0.004 : 1 และ 0.002 : 1 โดยปริมาตร ที่เวลากักเก็บ 6 ชั่วโมง และ 12 ชั่วโมง ตามลำดับ การใช้เทคนิคการส่องกล้องจุลทรรศน์พบ จุลชีพหลักในรูป แท่งสั้น กลม เส้นใย และเกรียวในถังกรองไร้ออกซิเจนที่ศึกษา ในส่วนของอาเคียที่ทำหน้าที่สร้างมีเทนพบจุลชีพหลักที่มีลักษณะคล้ายคลึงกับ *Methanosarcina* และ *Methanoseata* ผลของเจล DGGE พบการเกิดขึ้นและหายไปของแถบบนเจลที่ตอบสนองต่อการเพิ่มและลดลงของประสิทธิภาพของถังปฏิกรณ์ในการกำจัดฟอร์มัลดีไฮด์และฟีนอล ความแตกต่างของจำนวนแถบบนเจลที่พบ ยังแสดงให้เห็นถึงความหลากหลายของจุลชีพในถังกรองไร้ออกซิเจนที่บำบัดน้ำเสียที่ปนเปื้อนฟอร์มัลดีไฮด์และฟีนอลที่ความเข้มข้นต่างๆ กัน

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ปีการศึกษา 2552 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

5187569720: MAJOR ENVIRONMENTAL MANAGEMENT
KEYWORDS: EMBALMING FLUID/ FORMALDEHYDE/ PHENOL/ ANAEROBIC FILTER/ DOMESTIC WASTEWATER/ PHASE CONTRAST/ PCR-DGGE

WANAWAN PRAGOT: TREATMENT OF EMBALMING FLUID USING DOMESTIC WASTEWATER AS CO-SUBSTRATE IN ANAEROBIC FILTER. THESIS ADVISOR: PATIROOP PHOLCHAN, PH.D., 127 PP.

Simultaneous formaldehyde (FA) and phenol, the main ingredients of embalming fluid, biodegradation using domestic wastewater as the co-substrate in anaerobic filter was investigated. Experiments were conducted in two anaerobic filters operated at 6h and 12h HRT for 215 days. FA and phenol concentrations of 22-1,373 mg/l and 5.4-208 mg/l, corresponding to COD concentration of 207-1,756 mg/l, were fed to both reactors. FA removal efficiencies of 97.0% and 97.2% were obtained in the 6h and 12h reactors at all applied FA concentrations. On the other hand, phenol was observed to be completely removed when initial concentrations were not higher than 15 mg/l and 33 mg/l (with FA concentrations of 64 and 128 mg/l) in 6h- and 12h-HRT reactors, respectively. It was possible that high concentration of FA affected some microorganisms responsible in degrading phenol. Results obtained also indicated that anaerobic filter operated at longer HRT (12h) could achieve higher performance in removing studied toxic substances than that of the shorter one (6h). This could possibly be attributed to the decrease of microorganisms activity from the acute toxic of FA and phenol. Moreover, presence of embalming fluid in the influent caused the decrease of specific methane yield. The maximum treatable ratio of embalming fluid to domestic wastewater were found to be 0.004 : 1 and 0.002 : 1 by volume at the HRTs of 6h and 12h, respectively. Using the microscopic technique, dominant microorganisms were observed in the rods, cocci, filament, and helices forms. The organisms similar to *Methanosarcina* and *Methanoseata* were observed to dominate in all studies among the methanogenic archaea. DGGE profile revealed the appearances and losses of bands in response to the improvement and deterioration of reactor's performance in removing FA and phenol. Differences of the bands number were also visualized implying differences of microbial diversity in anaerobic reactors treating wastewater contaminated with different FA and phenol concentrations.

Field of Study : Environmental Management Student's Signature : Wanawan Pragot

Academic Year : 2009 Advisor's Signature: Patiroop Pholchan

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CONTENTS

Page

ABSTRACT (THAI) iv
ABSTRACT (ENGLISH) v
ACKNOWLEDGEMENTS vi
CONTENTS vii
LIST OF TABLES xi
LIST OF FIGURES xiii
LIST OF ABBREVIATIONS xv
CHAPTER I INTRODUCTION 1
1.1 Motivation 1
1.2 Objectives 3
1.3 Scope of this work 3
1.4 Benefits of this work 4
CHAPTER II THEORY BACKGROUND AND LITERTURE REVIEWS 5
2.1 Anaerobic process 5
2.1.1 The anaerobic treatment 5
2.1.2 The rationale for anaerobic treatment process 7
2.1.2.1 Advantages of anaerobic treatment process 7
2.1.2.2 Disadvantages of anaerobic treatment process 8
2.2 Basic concept of the reactor 8
2.2.1 Batch Reactor 8
2.2.2 Continuous Stirred Tank Reactor (CSTR) 9
2.2.3 Plug Flow Reactor (PFR) 10
2.3 Type of anaerobic reactor 11
2.3.1 Completely mixed reactor 11
2.3.2 Anaerobic contact process 11
2.3.3 Fluidized-bed reactor 12
2.3.4 Upflow anaerobic sludge blanket (UASB) 12
2.3.5 Expanded granular sludge bed (EGSB) reactor 12

Page

2.3.6 Anaerobic filter 13
2.3.6.1 Characteristic of media 13
2.3.6.2 Temperature 13
2.3.6.3 pH 14
2.3.6.4 Hydraulic retention time and organic loading rate 15
2.3.6.5 Nutrient in the wastewater 16
2.4 Characteristics of Embalming Fluid 16
2.4.1 Formaldehyde (FA) 16
2.4.1.1 Physical Characteristic of Formaldehyde 17
2.4.1.2 Toxicity of Formaldehyde 17
2.4.2 Phenol 17
2.4.2.1 Physical Characteristic of Phenol 17
2.4.2.2 Toxicity of Phenol 17
2.5 Co-substrate 18
2.6 Microbial characteristics of anaerobic process 19
2.6.1 Polymerase chain reaction (PCR) technique 20
2.6.2 Denaturing Gradient Gel Electrophoresis (DGGE) 21
2.7 Literature reviews 22
CHAPTER III METERIALS AND METHODS 27
3.1 Wastewater and inoculum 27
3.2 Embalming fluid 28
3.3 Anaerobic filter's media 28
3.4 Lab-scale anaerobic filter 29
3.5 Reactor operation and experimental setup 31
3.6 Physical and chemical analysis 32
3.6.1 Wastewater sampling and analysis 32
3.6.2 VFAs analysis 34
3.6.3 FA analysis 34
3.6.3.1 Preparation of calibration curve of FA 34
3.6.3.2 Sample measurement 35

3.6.4 Phenol analysis 35
3.6.4.1 Preparation of calibration curve of phenol 35
3.6.4.2 Sample measurement 36
3.6.5 Biogas composition analysis 36
3.7 Microbiological observations and analysis 37
3.7.1 Characterisation of biomass 37
3.7.2 Sample fixation 37
3.7.3 DNA extraction 37
3.7.4 PCR amplification 38
3.7.5 Denaturing Gradient Gel Electrophoresis (DGGE) 40
3.8 The Statistical analysis 40
CHAPTER IV RESULTS AND DISCUSSION 41
4.1 Physical and chemical analysis during start-up period 41
4.1.1 Temperature 41
4.1.2 pH 42
4.1.3 Alkalinity and Volatile Fatty Acid (VFA) 43
4.1.4 Suspended Solid (SS) and Volatile Suspended Solid (VSS) 44
4.1.4.1 Suspended Solid (SS) 45
4.1.4.2 Volatile Suspended Solid (VSS) 46
4.1.5 COD 47
4.1.6 Gas volume and gas composition 48
4.1.7 Profile of parameters along reactor's height. 50
4.2 Reactor performance after embalming fluid addition. 52
4.2.1 Temperature 56
4.2.2 pH and alkalinity 55
4.2.3 Suspended Solid (SS) and Volatile Suspended Solid (VSS) 58
4.2.4 COD 61
4.2.5 FA 63
4.2.6 Phenol 69
4.2.7 VFA composition 72
4.2.8 Gas volume and gas yield 74

4.2.9 The maximum treatable ratio of embalming fluid to domestic wastewater 77
4.3 Microbiological observations and analysis 77
4.3.1 The physical characterisation of biomass 77
4.3.2 DNA extraction 82
4.3.3 PCR 83
4.3.4 DGGE 84
4.4 Case study: Treatment of embalming fluid by using domestic wastewater as co-substrate in full-scale anaerobic filter: Chaing Mai University's case 89
CHAPTER V CONCLUSIONS 92
CHAPTER VI RECOMMENDATIONS FOR FUTURE WORK 94
REFERENCES 95
APPENDIX… 98
APPENDIX A CALIBRATION DATA AND CURVES 99
APPENDIX B EXPERIMENTAL DATA 103
BIOGRAPHY 127

LIST OF TABLES

Page

Table 3.1 Chemical properties of FA and phenol 28
Table 3.2 Detail of the experiment 31
Table 3.3 Wastewater sampling and analysis 32
Table 3.4 FA solution preparation 33
Table 3.5 Phenol solution preparation 34
Table 3.6 The primers used for amplification of 16S rDNA of Archaea and Bacteria in this study 39
Table 4.1 The profile of parameters collected from Reactors A and B 51
Table 4.2 Reactor performance obtained from every conduced experiment 53
Table 4.3 Influent and effluent pH and alkalinity observed in this study 57
Table 4.4 FA concentrations in samples taken from the influent tank during the 8 h period. 65
Table 4.5 The maximum FA concentration and corresponding parameters 66
Table 4.6 Comparism of FA removal efficiency found in this current study and in some previous studies removal efficiency 67
Table 4.7 The maximum phenol concentration and corresponding parameters 70
Table 4.8 Comparism of phenol removal efficiency found in this current study and in some previous studies removal efficiency 71
Table 4.9 Methane compositions in the biogas during whole experimental period 75
Table 4.10 FA and phenol concentrations of each maximum treatable ratio 77
Table 4.11 The microscopic observation of sludge 75
Table A-1 Calibration data of VFAs 97
Table B- 1 pH and temperature throughout the operation period 104
Table B- 2 Gas volume and gas yield throughout the operation period 109
Table B- 3 Alkalinity and VFA in the start up period 114
Table B- 4 SS throughout the operation period 114
Table B- 5 VSS throughout the operation period 115
Table B- 6 COD and FCOD throughout the operation period 117

Page

Table B- 7 Gas compositions throughout the experimental period 118
Table B- 8 FA throughout the operation period 119
Table B- 9 Phenol throughout the operation period 121
Table B- 10 Influent VFAs composition throughout the operation period 123
Table B- 11 Influent VFAs composition throughout the operation period 124

LIST OF FIGURES

Page

Figure 2.1 Anaerobic treatment process 6
Figure 2.2 The batch reactor 9
Figure 2.3 The CSTR 10
Figure 2.4 The Plug flow reactor 11
Figure 3.1 Domestic wastewater treatment plant at Chiang Mai University 27
Figure 3.2 Dimensions of bioball 29
Figure 3.3 Lab-scale anaerobic filter 30
Figure 3.4 Biogas collection in the 4 L plastic container 30
Figure 3.5 Biogas sampling 35
Figure 4.1 Temperature during the start up period 42
Figure 4.2 pH during the start up period 43
Figure 4.3 Alkalinity during the start up period 44
Figure 4.4 VFA during the start up period 44
Figure 4.5 SS concentration during the start up period 45
Figure 4.6 SS removal efficiency during the start up period 45
Figure 4.7 VSS concentration during the start up period 46
Figure 4.8 VSS removal efficiency during the start up period 46
Figure 4.9 COD concentration during the start up period 47
Figure 4.10 COD removal efficiency during the start up period 47
Figure 4.11 The volume of gas production during the star up period 48
Figure 4.12 The composition of gas production during the start up period 49
Figure 4.13 Partial pressure of CO_2 in biogas as a function of alkalinity for difference values of pH. 49
Figure 4.14 Specific methane yield (L of CH_4/ gCOD) during the start up period 49
Figure 4.15 The Reactor A profile in the start up period 51
Figure 4.16 The Reactor B profile in the start up period 52
Figure 4.17 The temperature during the whole experimental period 56
Figure 4.18 pH during the whole experimental period 57

Page

Figure 4.19 Alkalinity during the whole experimental period 58
Figure 4.20 SS concentration during the whole experimental period 59
Figure 4.21 SS removal efficiency during the whole experimental period 60
Figure 4.22 VSS concentration during the whole experimental period 60
Figure 4.23 VSS removal efficiency during the whole experimental period. 61
Figure 4.24 COD concentration during the whole experimental period. 62
Figure 4.25 COD removal efficiency during the whole experimental period. 62
Figure 4.26 FA concentration during the whole Experimental period. 64
Figure 4.27 FA removal efficiency during the whole Experimental period. 64
Figure 4.28 Phenol concentration during the whole experimental period. 69
Figure 4.29 Phenol removal efficiency throughout the operation period. 69
Figure 4.30 VFA composition 74
Figure 4.31 The volume of gas production during the whole experimental period. 75
Figure 4.32 Specific methane yield (L CH_4/gCOD removed) throughout the operation period. 76
Figure 4.33 Phase-contrast photomicrograph of bacteria and methanogenic archaea in biomass samples. 80
Figure 4.34 DNA extracted from the samples 82
Figure 4.35 The 1079 base pair PCR product from the archaea primer 46F/1100R compared with 100 base pairs DNA marker 83
Figure 4.36 The 179 base pairs PCR product from the archaea primer F340GC/ R519 compared with those of the 100 base pairs DNA marker 84
Figure 4.37 The 149 base pairs PCR product from the bacteria primer F341GC/ R534 compared with 100 base pairs DNA marker 84
Figure 4.38 DGGE profile of archaea communities from Reactor A 85
Figure 4.39 DGGE profile of archaea communities from Reactor B 86
Figure 4.40 DGGE profile of bacteria communities from Reactor A 88
Figure 4.41 DGGE profile of bacteria communities from Reactor B 89
Figure A-1 Calibration curve data of VFAs 101

LIST OF ABBREVIATIONS

ASBBR	Anaerobic Sequencing Batch Biofilm Reactor
AWWA	American Water Works Association
°C	Degree Celsius
$CaCO_3$	Calcium Carbonate
cm	Centimeter
C_6H_5OH	Phenol or carbolic acid
CH_4	Methane
CH_3CH_2COOH	Propiomic acid
$CH_3CH_2CH_2COOH$	Butyric acid
CHOH	Formaldehyde
cm^2/cm^3	Square centimeter/Cubic centimeter
COD	Chemical oxygen demand
CO_2	Carbon dioxide
CO_3^{2-}	Carbonate
CTAB	Cetyltrimethylammonium Bromide
CSTR	Continuous Stirred Tank Reactor
d	day
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic Acid
EDTA	Disodiumethylenediamine Tetraacetate Dehydrate
EGSB	Expanded granular sludge bed
EPA	Environmental Protection Agency
FA	Formaldehyde
g/cm^3	Gram per Cubic Centimeter
g/L	Gram per Liter
$g/m^3.h$	Gram per Cubic meter. hour
$g/m^3.d$	Gram per Cubic meter. day
g/mol	Gram per Molar
GC	Gas Chromatograph
H_2	Hydrogen gas

HCO_3^{2-}	Bicarbonates
H_2S	Hydrogen sulfide
hr	Hour
HRT	Hydraulic Retention Time
K	Potassium
kgCOD/m^3.d	Kilogram Chemical oxygen demand per Cubic Meter. Day
kgFA/m^3.d	Kilogram formaldehyde per Cubic Meter. Day
kgPhenol/m^3.d	Kilogram phenol per Cubic Meter. Day
kWh	kilowatt. hour
µl	Micro liter
L	Liter
L/d	Liter per day
µm	Micrometer
m	Meter
$MgCl_2$	Magnesium Chloride
mg/l	Milligram per Liter
min	Minute
m^2/m^3	Square meter/Cubic meter
mM	Milimole
O_2	Oxygen
OH^-	hydroxide
P	Phosphorous
PCR	Polymerase chain reaction
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
s	Second
SBR	Sequencing batch reactor
SRT	Solids Retention Time
N	Nitrogen
NH_3	Ammonia
NaCl	Sodium Chloride
UASB	Upflow Anaerobic Sludge Blanket

USEPA	United States Environmental Protection Agency
UV	Ultraviolet Absorbtion
VFAs	Volatile Fatty Acids
VOC	volatile organic compound
w	week

CHAPTER I

INTRODUCTION

1.1 Motivation

In order to comply with more stringent environmental regulations, it is compulsory for almost all wastewater generators to treat wastewater before released to the environment. Problems, however, occurred to some wastewater generators, e.g. chemical laboratories, hospitals, because several toxic compounds are used in their activities. Among chemical compounds used in some large scale hospitals, Embalming fluid is a common compound used in disinfection and preserves biological materials and, therefore, utilised in relatively large amount. Embalming fluid, the combination of formaldehyde (FA) and phenol, contaminated in the wastewater could adversely affect microorganisms used in the treatment process, resulting in the treatment system being upset.

FA is commonly used in making preservatives, disinfectants, and antiseptics. A 0.5% FA solution destroys all species of microorganism in a period of 6-12h (Oliveira et al., 2004). Formaldehyde can damage DNA, RNA and protein directly through cell at low concentrations and cause the death of microorganisms (Lu and Hegemann, 1998). Moreover, this chemical was found to be carcinogenic when exposed at high concentration. The toxicity of FA ranks in the first place of chemical discharged by industries (Edwards, 1999). However, FA is known to be biodegradable in both aerobic and anaerobic systems. Anaerobic treatment is more appropriate; however, because of its low energy consumption and small sludge production. Many types of reactors including batch reactor (Lu and Hegemann, 1998), fluidized bed bioreactor (Moteleb et al., 2002), horizontal flow anaerobic immobilized sludge reactor (Oliveira et al., 2004), upflow anaerobic fixed film reactor (UAFB) (Raja Priya et al., 2009) and anaerobic sequencing batch biofilm reactor (Pereira and Zaiat, 2009) have been utilised in the studies of FA biodegradation. Results obtained from these studies indicated that higher FA concentration could be applied to the system

when microorganisms were given longer time to acclimatise. However, inhibition, toxicity and accumulation of volatile fatty acid were found in batch reactors treating FA. Continuously fed plug flow reactors were reported to achieve better FA removal performance. Moreover, the degradation was found to be greatly enhanced in the presence of a co-substrate (Omil et al., 1999).

Phenol, a simple aromatic chemical, is toxic to most microorganisms and is commonly used as a general disinfectant (Udomsinroj, 2003). It is water soluble and highly mobile, and likely to reach drinking water sources downstream from discharges. Even at low concentrations, it can cause severe odor, taste problems and poses risks to exposed organisms. As a toxic and potentially carcinogenic chemical, release of phenolic compounds into the environment is of great concern (ATSDR., 1989). Therefore, elimination of these compounds is a necessity to preserve the environmental quality. Anaerobic biological process has been considered as an alternative treatment thanks to the reported capability of microorganisms in biodegrading phenol. (Fang and Chan, 1997) observed phenol's effects on cell activity when increased phenol loading rate at low HRT in UASB reactor. Eiroa, et al. (2005) indicated that phenol caused inhibition and an acclimation period of the sludge was necessary. In the presence of 260 mg/l formaldehyde, complete phenol removal took place only in assays with initial concentrations of 30 - 180 mg/l after formaldehyde was completely removed. However, in the horizontal-flow anaerobic immobilised biomass reactor, the toxicity and inhibition problem caused by phenol were not observed. This type of reactor was successfully used in the treatment of synthetic wastewater containing phenol (Bolanos et al., 2001).

In the previous studies, various types of anaerobic reactors were reported to satisfactorily treat FA or phenol (Fang and Chan, 1997; Qu and Bhattacharya, 1997; Lu and Hegemann, 1998; Gonzalez-Gil et al., 1999; Omil et al., 1999; Vidal et al., 1999; Lotfy and Rashed, 2002; Moteleb et al., 2002; Fang et al., 2004; Eiroa, 2005; Fang et al., 2006; Scully et al., 2006; Agarry et al., 2008; Hernandez and Edyvean, 2008; Pereira and Zaiat, 2009; Raja Priya et al., 2009). However, utilizing anaerobic filter in treating these compounds has shown to be more effective because no inhibition problem was generally observed (Bolanos et al., 2001; Oliveira et al., 2004). In the biological process, the knowledge of microbial community diversity and

its functions with the parameters affecting the growth of the organisms present can be very useful. The development of the molecular biology tools has contributed to the detection, quantification, and identification of the microbial communities involved in the treatment reactor. In addition, denaturing gradient gel electrophoresis (DGGE) techniques are an appropriate tool for comparison and quantification of the changes in the microbial composition (Ke et al., 2008).

To our knowledge, the study of simultaneous removal of both FA and phenol using domestic wastewater as the co-substrate is still lacking. The aim of this study was, therefore, to investigate performance of anaerobic filter in removing FA and phenol in the synthetic embalming fluid using domestic waste as co-substrate. Maximum amount of embalming fluid, which could be efficiently treated with domestic wastewater, was determined. In addition, the microbial communities functioning inside the reactor were also explored using both the microscopic and PCR-DGGE techniques to link between their patterns and reactor performance.

1.2 Objectives

The main objective of this study was to investigate performance of anaerobic filter in removing FA and phenol in the synthetic embalming fluid using domestic wastewater as co-substrate. The specific objectives were:

1. To study effects of organic loading rate and hydraulic retention time on the anaerobic filter performance during removal of formaldehyde and phenol, the main ingredients of embalming fluid.
2. To determine the maximum amount of embalming fluid in domestic wastewater that can be treated by anaerobic filter.
3. To investigate the microbial communities growing in anaerobic filter operated at different conditions on degradation of formaldehyde and phenol.

1.3 Scope of this work

1. All experiments were conducted using 2 identical anaerobic filters made from the PVC pipe with the diameter of 10 cm and 160 cm in height. Of the 10.2 l total volume, the upper 7.1 l was allocated for packing media

and the lower 3.1 l under the media bed was allocated for suspended cultures (reactor depth without media is 30% of the total working depth).

2. The media used was the bioball with the diameter of 4 cm, void ratio 90% and specific surface area of 307 m^2/m^3
3. Two anaerobic filters were operated at different HRT, i.e. 6 h- and 12 h-HRT.
4. The domestic wastewater used in this study was collected from an equalisation tank of Chiangmai University wastewater treatment plant.
5. The embalming fluid was synthesised from water, formaldehyde and phenol at the ratio of 100: 4: 1 by volume. This ratio were obtained from the real situation used.

1.4 Benefits of this work

1. Knowing the maximum amount of embalming fluid in domestic wastewater that can be treated by anaerobic filter.
2. The optimum values of organic loading rate and hydraulic retention time can be used in the design and operation of anaerobic filter in treating embalming fluid.
3. Understanding the role of microorganisms/ microbial communities in degrading embalming fluid.

CHAPTER II

THEORY BACKGROUND AND LITERTURE REVIEWS

2.1 Anaerobic process

2.1.1 Anaerobic treatment

Anaerobic treatment is the biological treatment without the use of air or elemental oxygen. In anaerobic treatment organic pollutants are converted by anaerobic microorganisms to biogas which are methane (CH_4), carbon dioxide (CO_2) and other products as showed in Equation 2.1 (Eddy, 2004).

$$\underset{\text{(Organic substances)}}{\text{CHONS}} \longrightarrow CH_4 + CO_2 + H_2 + NH_3 + H_2S + \text{Other products} \quad \text{(Eq.2.1)}$$

The overall anaerobic conversion of biodegradable organic materials to final end products, methane and carbon dioxide, is occurred from the co - operation of two types of bacteria ; acid forming or non – methanogenic bacteria and methanogenic bacteria. Anaerobic treatment comprises four steps (Figure 1) which occurred four steps in order as followed;

Step 1 Hydrolysis

Large organic matter molecules, i.e. carbohydrate, protein and fat, are hydrolised into their simple monomer compounds such as glucose, amino acid and some fatty acids. This process is mediated by extracellular enzymes produced by microorganisms.

Step 2 Acidogenesis

The simple monomer compounds from the hydrolysis step are degraded further to volatile fatty acid such as propionic, butyric, valeric and acetic acid.

Step 3 Acetogenesis

The volatile fatty acids from the acidogenesis step are transformed by acid forming bacteria and hydrogen forming bacteria to acetic acid, hydrogen gas (H_2) and carbon dioxide (Eq. 2.2 and 2.3).

$CH_3CH_2COOH + H_2O \longrightarrow CH_3COOH + CO_2 + H_2$ (Eq.2.2)

(Propionic acid)

$CH_3CH_2CH_2COOH + 2H_2O \longrightarrow 2CH_3COOH + 2H_2O$ (Eq.2.3)

(Butyric acid)

These reactions are brought about by the facultative bacteria and the obligate bacteria both of which known as acid formers or non – methanogenic bacteria. During this step the pH of the system decreases because the production of acid by these bacteria.

Step 4 Methanogenesis

Finally, methane producing bacteria, known as methanogenic bacteria, convert acetic acid and hydrogen gas produced in the acetogenesis step to final products which are mainly CH_4 and CO_2. This step is called the methanogenic phase or methanogenesis. These reactions (Equation 2.4 and 2.5) are also known as methane formation.

$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O$ (Eq.2.4)

$CH_3COOH \longrightarrow CH_4 + 2H_2O$ (Eq.2.5)

The accumulation of acetic acid and hydrogen gas from the previous step can affect methane formation, as the methanogenesis bacteria cannot survive in acidic conditions (Raja Priya et al., 2009).

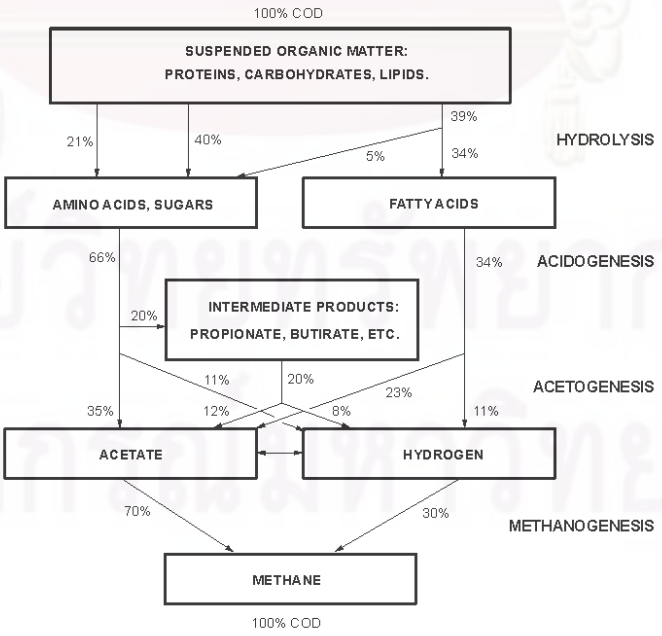


Figure 2.1 Anaerobic treatment process (Van Haandel, 2007)

2.1.2 The rationale for anaerobic treatment process

The rationale for anaerobic treatment process can be explained by considering the advantage and disadvantages of this process.

2.1.2.1 Advantages of anaerobic treatment process

- Less energy required

Anaerobic process is the net energy producer instead of energy user, as in the case of aerobic process. The anaerobic treatments need no air supply. In contrast with the aerobic process requires energy in aeration step. On the other hand, the anaerobic process produces methane which is the source of energy. Aerobic treatment are energy-intensive process for the removal of organic matter, requiring 0.5-0.75 kWh of aeration energy for 1 kg of COD removed (Adrianus et al., 1994).

Low production of biomass

Anaerobic treatment processes utilise more than 90% of the biological degradable organic matter (COD) for methane production, with only 10% or less converted to biomass. Because of the relatively lower growth rate of anaerobic microorganisms, the sludge were produced small amount. Aerobic treatment process, generates considerable amounts of sludge. Biological oxidation of every kilogram of soluble BOD produces 0.5 kg of sludge. The costs of treatment and disposal of sludge account for 30-60% of the total operational costs in a conventional activated sludge process.

- Smaller reactor volume required

The volumetric organic loading rates normally used for that anaerobic process are 5-10 times higher than for aerobic process (Speece, 1996), so smaller reactor volumes and less space may be required for treatment. The large volumetric organic loading rate can be applied. Moreover, the land requirements for the anaerobic treatment unit were reduced.

- Low nutrient requirement

Owing to the lower biomass synthesis rate during the anaerobic process, the nutrient requirements are considerably lower, with the anaerobic process requiring just 20% of the nutrients required for the aerobic process. The cost for nutrient

addition is much lesser in anaerobic process for anaerobic process because less biomass is produced.

- Ability to reduce concentrations of refractory organics

With proper acclimation, many of the previously identified refractory organics such as carbon tetrachloride, chloroform, trichloroethylene, formaldehyde, and phenol have been successfully transformed to a lower toxic by anaerobic microorganisms (LaGrega et al., 2006).

2.1.2.2 Disadvantages of anaerobic treatment process

- Operation consideration

Anaerobic processes require long start-up time, their sensitivity to possible toxic compounds, operational stability, the potential for odor production, and corrosiveness of the digester gas are considered to be problematic. However, with proper wastewater characterisation and process design these problems can be avoided and/or managed.

- Need for alkalinity addition

Alkalinity in wastewater results from the presence of hydroxide (OH^-), carbonates (CO_3^{2-}) and bicarbonates (HCO_3^{2-}). The alkalinity in wastewater helps to resist changes in pH cause by the presence of acid. Alkalinity concentration of 2000 to 3000 mg/l as $CaCO_3$ may be needed in anaerobic process to maintain an acceptable pH with the high gas phase CO_2 concentration (Metcalf&Eddy, 2004).

2.2 Basic concept of the reactor

2.2.1 Batch reactor

Batch reactors are widely used in many industries, especially in handling relatively small volume productions of very expensive materials such as enzymes, medicines and etc., because the same batch reactor can be used to produce a variety of products. Sequencing batch reactors (SBR) have also been proposed for wastewater treatment. This batch or sequencing batch process is still used in some very small installations, although it is rare.

In batch reactor experiments, the reactor is filled with the reactant and brought to the desired reaction conditions. Then, during the reaction, samples are analyzed and the concentration is recorded against time. When more than one reactant is involved, it is necessary to repeat the experiment with different initial compositions. In a batch reactor, there is no flow, instead a batch of material is placed into a vessel, inoculated, and microbial growth and substrate utilization occur. As growth proceeds, reaction conditions change and consequently so does the growth environment. The microorganisms present at different times will be in different physiological conditions and no steady state is possible, which makes modeling the batch system much more complicated.

Either the differential or the integral method must be used with data obtained from a batch reactor because the data are in the form of concentration versus time and do not provide a direct measure of the reaction rate as a function of concentration.

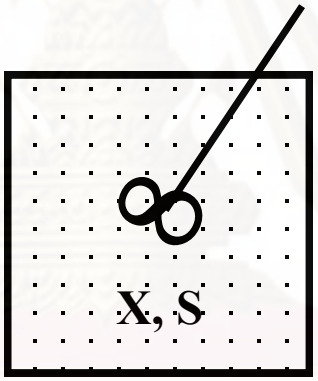


Figure 2.2 The batch reactor
(X: biomass , S: substrate)

2.2.2 Continuous Stirred Tank Reactor (CSTR)

A CSTR, also known as continuous flow stirred tank reactor (CFSTR) or completely mixed reactor, is used very frequently in many industrial fields. It is usually equipped with baffles and a mixer which is operated at a sufficiently high speed so that the mixing is assumed to be perfect. It is assumed to be homogeneous and instantaneous so that any reactant carried into the reactor by the feed is dispersed evenly throughout the reactor without any time delay. In addition, the reaction is assumed to take place only in the reactor so that the effluent composition is the same as the reactor composition.

The most often used method to get mathematical expression of biokinetics is steady state operation, where feed is supplied continuously until a steady state is achieved. Then, effluent concentration is recorded, and another steady state run should begin by changing the feed concentration and/or the feeding rate. Thus a number of steady state runs are required to obtain data relating reaction rate to concentration whereas a single unsteady-state run may be used to gain the same information from a batch reactor. By varying independent variables such as flow rate and/or influent substrate concentration, it is possible to solve mathematical expressions experimentally.

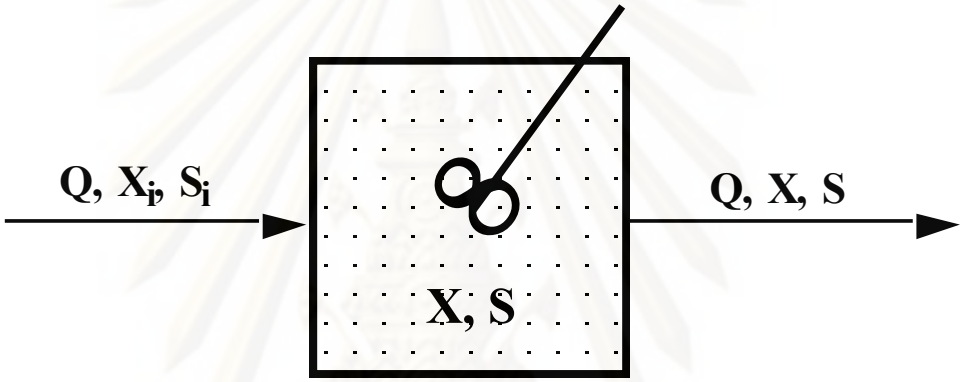


Figure 2.3 The CSTR

(Q : flow, X_i : influent biomass, S_i : influent substrate, X : biomass, S : substrate)

2.2.3 Plug Flow Reactor (PFR)

Fluid containing the substrate continuously passes through the reactor and effluent is discharged in the same sequence in which it enters. This type of flow is similar to that in long tubes or tanks with a high length to width ratio. For an ideal PFR, the flow pattern inside has uniform velocity and concentration in the radial direction at any point along the length of the reactor while longitudinal (axial) dispersion is minimal or absent.

In the ideal PFR, concentration of substrate and biomass continuously varies with time and the distance. In other words, it is not only necessary to know how the concentration varies with time but also how it varies along the length of the reactor.

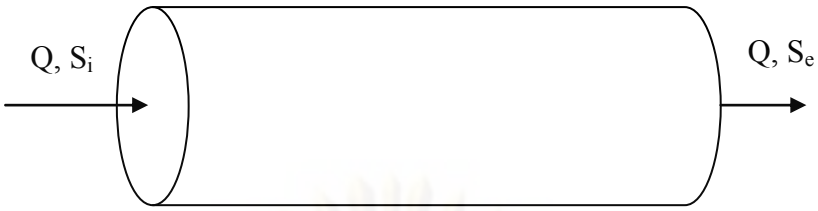


Figure 2.4 The plug flow reactor
(Q : flow, S_i : influent substrate, S_e : effluent substrate)

2.3 Type of anaerobic reactor

2.3.1 Completely mixed reactor

In the completely mixed reactor, it is assumed that complete mixing occurs instantaneously and uniformly throughout the reactor as fluid particles enter the reactor. The completely mixed digester without sludge recycle is more suitable for waste with high concentrations of solids or extremely high dissolved organic concentrations. Where thickening the effluent is difficult, it is more practical to operate with SRT (solids retention time) in the range of 15 to 30d (Parkin and Owen, 1986).

2.3.2 Anaerobic contact process

Anaerobic contact process, an analogy to the aerobic activated sludge system, provides for separation of seed organisms. Many problems are occurred in anaerobic contact process; one is the biomass in the settling tank to rise due to bubble generation and float in the settling tank (Speece, 1996). Moreover, biomass loss to the effluent is a severe problem because the quantity of microorganisms produced is so much lesser than the aerobic process. Hence, the small biomass loss can significantly affect process stability, as well as effluent quality. To solve this problem, a degasifier is usually needed to minimised floating biomass in the separation step.

2.3.3 Fluidized-bed reactor

The fluidized-bed reactor contains small media, such as sand, coal, granular activated carbon, polyurethane foam, fine clay and porous glass, which bacteria attach (Chernicharo, 2007). The small media size gives a very high specific surface area for

biofilm. In the fluidized-bed reactor, wastewater is pumped upward through sand. The high upward velocity should be maintained by recycle the effluent to mix with the influent. The advantage of the fluidized-bed reactor has been found in the use of granular activated carbon as the support for the treatment of wastewater containing toxic. On the other hand, high recycle rate can incur energy costs, mainly due to head loss in the recycle piping.

2.3.4 Upflow anaerobic sludge blanket (UASB)

The UASB is the reactor suitable for the treatment of wide range of wastewater, including the industrial wastewater and dilute municipal wastewater (Metcalf&Eddy, 2004). Wastewater flows upward from the bottom of the reactor, where it must be distributed uniformly. Methane and carbon dioxide gas rise and captured in the gas dome at the top of the reactor. The solids return to the bottom area while the liquid exits over the weirs on the top. Formation of sludge granules and theirs maintenance is extremely important in the operation of this process.

2.3.5 Expanded granular sludge bed (EGSB) reactor

The EGSB reactor is the variation of the fluidized-bed reactor and UASB reactor. It contains similar support media to the fluidized-bed reactor and UASB reactor. The difference is that the upward velocity in EGSB is not maintained as high as in the fluidized-bed reactor and as low as UASB reactor. The EGSB design is appropriate for low strength soluble wastewaters (less than 1 to 2 g soluble COD/l) or for wastewaters containing inert or poorly biodegradable suspended particles which should not be allowed to accumulate in the sludge bed (McCarty, 1986).

2.3.6 Anaerobic filter

Anaerobic filter is a type of bioreactor used in the treatment of wastewater. It contains the media having high specific surface area for bacteria adhesion (Tay, 1999). Wastewater can be fed to the reactor either in the up-flow or down-flow mode. Factors affecting the performance of anaerobic filter can be presented as;

2.3.6.1 Characteristic of media

Anaerobic filter contains the media with high specific surface area to achieve high retention of biomass for efficient and stable operation. Many biofilter companies have proprietary media that are designed to provide optimal performance through optimising: high surface area for growth, long-term physical stability, low pressure drop, pH buffer capacity and nutrients. However, high surface area media can be easily clogged up. This problem can be prevented by using media with higher void ratio. It has been reported that high void ratio media have higher COD removal efficiency (Tay, 1998).

The height of packing media inside anaerobic filter should be in the range of 0.8 – 3.0 m depending on the flow direction, type of media and the influent concentration to prevent the obstruction of bed (Chernicharo, 2007). However, suspended biomass growing in the media void has been found to be the most active microorganisms in degrading organic compounds (Tay, 1999). If the hybrid reactor is to be utilised, total depth of anaerobic filter to the depth of reactor without media should be in the range of 30 – 50 percent (Metcalf&Eddy, 2004).

2.3.6.2 Temperature

As stated previously, degradation of organic substances in wastewater is mediated by the co-operation of non-methanogenic bacteria and methanogenic bacteria. The methanogenic bacteria control the degradation rate because they are sensitive to temperature level. Temperature control is also very important in anaerobic filter to avoid thermal shock. The effect of temperature shocks on reactor performance depends upon factors such as the exposed temperature, duration of shock, sludge characteristics and imposed specific sludge load. At temperatures exceeding that of the maximum growth, the decay rate will generally exceed the bacterial growth rate, and consequently a decrease in specific sludge activity and reactor efficiency may occur (Van Lier et al., 1990). According to Borja and Banks (1995), a shock change in temperature may be characterised by an immediate pH drop in the reactor, which then would stabilise at a value slightly below the previous steady state pH value. This drop in pH is due to an increase of the mixed liquor (effluent) VFA-concentration,

which tends to approach a new level during operation at a reduced temperature. The effluent COD increases due to the increase of effluent VFA concentration and suspended solids (SS), as well as to the presence of components in the influent which remain un-converted. On the other hand, according to the results of Rintala and Lepisto (1997), who conducted the methanogenic activity test with thermophilic sludge (55˚C) at temperatures of 35, 50, 55, 58, 65, and 70 ˚C, there was some methane production during the first hours of the tests at 65–70 ˚C. However, this production slowed down and/or stopped 30 h later. No significant methane production was found at 35 ˚C until the end of the test, 70 h later. In addition, anaerobic degrading is found to function well under mesophilic condition (30 – 35 ˚C) and thermophelic condition (48 – 57 ˚C). Bioactivity roughly doubles for each 10 ˚C. (Speece, 1996).

2.3.6.3 pH

It is well known that methanogenic activity is more likely to proceed optimally in a narrow pH value range, between 6.3 and 7.8(Van Haandel A., 2007). The effect of a drastic pH-change in the influent depends on the available alkalinity in the reactor. Tests carried out by Borja and Banks (1995) showed that during a 10-h period, neither an influent pH of 10 nor an influent pH of 3 significantly affected the reactor stability. This was because the buffer capacity of the system sufficed to maintain the pH of the medium in the reactor in the optimal range. In experiments dealing with the treatment of a synthetic wastewater containing VFA and sulphate, Visser et al. (1993) concluded that methanogenesis was inhibited at a medium-pH exceeding 8, which then resulted in the development of a sludge dominated by sulphate-reducing bacteria. They also concluded that sulphate-reducing bacteria are less sensitive to short-term (8 h) pH variations than methanogenic bacteria. Moletta et al. (1994) tested an on-line automatic system for pH control of an anaerobic fluidised-bed reactor. Some of the tests applied to the system can be useful for elucidating what occurs to an anaerobic reactor during a small change in pH. They first injected HCl to lower the reactor pH from 6.8 to 6.6, and found an immediate response, viz. the gas production increased by 40%, as well as the concentration of CO_2 in the biogas. The hydrogen content remained almost unchanged. They also tested the reactor by adding

NaOH to increase the reactor pH up to 7.4, and observed that the gas production increased, but the CO_2 concentration substantially decreased. The variations in the gaseous phase were the consequence of a shift in CO_2 solubility with pH. According to Lettinga et al. (2000), based on experimental results obtained with sugar beet wastewater, process efficiency recovers almost immediately from pH shocks once the influent pH is returned to the optimal range. In the case of sudden drastic changes, the recovery of the process depends on the extent and duration of the imposed change, as well as on the concentration of volatile fatty acids during the event. It was concluded that decrease of pH from the accumulation of VFAs adversely affected the performance of methanogens in producing methane.

2.3.6.4 Hydraulic retention time and organic loading rate

Strong variations in flow and concentration may adversely affect the efficiency of an anaerobic reactor. The effect of hydraulic and organic load generally depends on the applied hydraulic retention time (HRT), sludge retention time (SRT), intensity and duration of the variations, sludge properties and the reactor design. The accumulation of volatile fatty acids (VFA) can be a typical reactor response during overloading, and during sudden variations in hydraulic and organic loading rates. Hydrogen partial pressure plays an important role in controlling the proportion of the various intermediate products of the anaerobic reactions. Under high H_2 in the reactor conditions, there may be a shift in the metabolic pathway to a less favorable route, resulting in a ratio shift between VFA producers (acidogens and acetogens population) and consumers (methanogens). Such a highly undesirable situation could lead to the production less amounts of carbon dioxide and hydrogen gas in the biogas. The partial pressure of hydrogen gas inside the reactor might increase to values exceeding 10^{-4} atm, which may then cause a shift in the metabolic pathway. When slowly growing methanogens cannot sufficiently and rapidly eliminate all H_2 produced by the H_2 producing bacteria (e.g. in case the sludge contains insufficient hydrogen consuming organisms), this may result in a distinct inhibition of the degradation of propionate, butyrate and lactate (Leitao et al., 2006). The suitable range of hydraulic retention time and organic loading rate for treating domestic wastewater

in anaerobic filter are between 5 – 10 hours and 0.15 – 0.50 kgBOD/m^3.d or 0.21 – 0.71 kgCOD/m^3.d, respectively (Chernicharo, 2007).

2.3.6.5 Nutrients in the wastewater

Nutrients, carbon and energy required for microorganism may derive from the component of the wastewater. Wastewater suitable for being treated in anaerobic filter should have COD : N : P ratio at least 100 : 1.1 : 0.2 and low level of suspended solid to prevent media clogging. Phophorus (P) is directly involved in biosynthesis, whereas nitrogen (N) is involved in the energy transfer system of microorganisms. Moreover, the wastewater should have adequate amount of micro – nutrient, e.g. Fe, Co, Ni, SO_4^{2-}, for bacteria to maintain their activities (Tuntoolavest, 1995).

2.4 Characteristics of embalming fluid

Embalming fluid is a colorless liquid used in medical process for disinfection, fungicide and preserve biological materials. This liquid has acute effects to skin, eyes, and nose, if exposed in high concentration. Generally, embalming fluid contains the mixer of water, formaldehyde, and phenol at the ratio of approximately 100 : 4 : 1 by volume. However, embalming fluid could be treating via biological process, especially utilizing anaerobic process in treating these compounds has shown to be more effective because no energy required. The details of both compounds were showing below.

2.4.1 Formaldehyde (FA)

Formaldehyde (CHOH) or methyl aldehydes, a chemical in aldehydes group, is commonly used in making preservatives, disinfectants and antiseptics in the hospital and chemical laboratory. A 0.5% formaldehyde solution destroys all species of microorganisms within a period of 6–12 h (Oliveira et al., 2004). Many toxic substances are found as contaminants in formaldehyde, such as formic acid, acetic acid, methanol, phenol etc. Formaldehyde is reported to be in the first place of the ranking of 643 chemicals having environmental impacts discharged by industries (Edwards, 1999).

2.4.1.1 Physical characteristic of formaldehyde

Formaldehyde is a colorless liquid causing irritant and inflammable fume. This is because this compound is a volatile organic compound (VOC), which becomes a gas at normal room temperatures. It has density of 1.09 g/cm^3 and flashing point =56 °C, melting point = -92 °C and boiling point = -21 °C (Udomsinroj, 2003).

2.4.1.2 Toxicity of formaldehyde

Formaldehyde irritatings tissues upon direct contact. Formaldehyde gas causes a burning to the eye, nose, and lung. Moreover, formaldehyde can damage DNA, RNA and protein directly through cell at low concentrations causing cancer in human and death of microorganisms (Lu and Hegemann, 1998).

2.4.2 Phenol

Phenol or carbolic acid (C_6H_5OH) is the chemical product synthesised from aromatic and hydroxyl (-OH) compounds. This chemical product is utilised in disinfection and medical applications, thus the wastewater containing phenol can have adverse effects to environment. Phenol is ranked in 481 from the list of 1,467 hazardous substances by Environmental Protection Agency (EPA) (ASTM, 2003).

2.4.2.1 Physical characteristic of phenol

Phenol is the colorless to brown crystal solid with specific odor, that have molecular weight =94.11 g/mol, density = 1.0576 g/cm^3, flashing point = 79 °C, melting point = 40.9 °C, boiling point = 182 °C, and pKa = 10.0. Phenol is soluble in water, glycerol, carbon sulfide, alcohol, ether and chloroform (ATSDR., 1989).

2.4.2.2 Toxicity of phenol

Phenol can be taken through inhalation, oral, and dermal routes of exposure. Moreover phenol suspended in the air can also be absorbed though skin. Once absorbed, phenol is rapidly distributed throughout the organs, especially liver and kidney which generally have the greatest amount of phenol. Ingestion of high

amounts of phenol or exposure a relatively high amounts of phenol on skin can cause alterations to the cardiac rhythm (ATSDR., 1989).

Standard of FA and phenol concentrations in different sources of water were promulgated by responsible organization in Thailand is presented in **Table 2.1**.

Table 2.1 Standard of FA and phenol concentrations in different sources of water were promulgated by responsible organization in Thailand.

Type of water	FA (mg/l)	Phenol (mg/l)
Drinking water	-	0.001
Industrial effluent	1	1

(Source: http://www.pcd.go.th)

2.5 Co-substrate

An interesting option for improving yields of anaerobic degradation is co-substrate. Co-substrate is the simultaneous digestion of more than one type of waste in the same unit (Agunwamba, 2001). Advantages include better digestibility, enhanced biogas production / methane yield arising from availability of additional nutrients, as well as a more efficient utilisation of equipment and cost sharing. In addition, economic advantages derived from the fact of sharing equipment are quite significant. Tay et al. (2001) also studied granulation with 1000 mg/l glucose as a co-substrate. They found that the reactor supplemented with glucose had a relatively shorter startup and granulation period (4 months, compared to 7 months for the reactor without glucose), and a larger granule size.

Phenol concentrations greater than 500 mg/L can be effectively treated with acclimatisation of inoculate, recirculation of the treated effluent and / or supplementing with co-substrates such as glucose, VFA and dilute molasses (Veeresh et al., 2005). According to Tay et al. (2000), the treatment of wastewaters containing phenolic compounds as the sole substrates at high concentrations in once-through UASB systems has met with several drawbacks. The inhibitory and toxic nature of phenolic compounds at high concentrations, even to granular sludge grown on phenol, prohibits further enhancement of organic load with desired performance. Relatively long acclimation period, small granule size and decrease in phenol removal efficiency

at higher loadings, sensitivity to temperature and loading shocks, and long recovery periods after shocks are a few problems associated with the treatment of phenol at high concentration. These problems, in principle, can be overcome by dilution through effluent recirculation and/or supplementing with a co-substrate.

2.6 Microbial characteristics of anaerobic process

Different groups of anaerobic microorganisms are needed for the degradation of one component – all of them dependent on the proper ‘‘functioning’’ of the others. Methanogenic bacteria which are assigned to the domain Archaea are responsible for the conversion of CO_2, H_2, and acetate into CH_4. Through their strict anaerobic growth and often syntrophic interactions, conventional cultivation is at least difficult. Due to their slow growth, methanogens might not be responsive fast enough regarding changes in substrate composition and are therefore often thought to be the limiting step of the whole fermentation. Also, the oxidation of fatty acids like butyrate or propionate can only occur when the H_2 built during the acetogenic phase is efficiently removed by methanogens. Many factors seem to influence the fermenter performance, e.g. the concentration of organic acids and chemical properties of the substrate material. Nevertheless, the role of many microorganisms involved in the whole process is still unknown or poorly investigated. It is essential to light up this black box for a better understanding of fermentation processes and therefore to be able to counteract in advance to unfavorable changes in reactor performance, thus making fermentation more energy-efficient. Their relationships to one another and to other microbes remain virtually unknown. Protein and nucleic acid primary structures are perhaps the most reliable indicators of phylogenetic relationships. By using molecule-microbiological techniques, such as the PCR-DGGE, it is possible to present the microbial populations in anaerobic process (Ke et al., 2008).

All living cells contain two type of nucleic acid which are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is double stranded helical molecular, where each strand is containing four difference nucleotide bases attached. The four difference nucleic acid consisted of adenine (A), guanine (G), cytosine (C) and thymine (T). Whereas, RNA contain only one strand and similar to the one strand of DNA, with difference bases thymine (T) in DNA is replaced by uracyl (U). There are

three major types of RNA, messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA).

For the analysis of natural microbial populations, in which unknown diversity must be anticipated, there are several reasons to focus on the rRNAs (Olsen et al., 1986).

- The rRNAs, as key elements of the protein-synthesizing machinery, are functionally and evolutionarily homologous in all organisms.
- The rRNAs are ancient molecules and are extremely conserved in overall structure. Thus, the homologous rRNAs are readily identifiable, by their sizes.
- Nucleotide sequences are also conserved. Some sequence stretches are invariant across the primary kingdoms, while others vary. The conserved sequences and secondary structure elements allow the alignment of variable sequences so that only homologous nucleotides are employed in any phylogenetic analysis. The highly conserved regions also provide convenient hybridization targets for cloning the rRNA genes and for primer directed sequencing techniques.
- The rRNAs constitute a significant component of the cellular mass, and they are readily recovered from all types of organisms for accumulation of a data base of reference sequences.
- The rRNAs provide sufficient sequence information to permit statistically significant comparisons.
- The rRNA genes seem to lack artifacts of lateral transfer between contemporaneous organisms. Thus, relationships between rRNAs reflect evolutionary relationships of the organisms.

There are three rRNAs types in bacteria composed of difference amount of nucleotides, 5S (120 nucleotides), 16S (1600 nucleotides), and 23S (3000 nucleotides) (Olsen, 1986). From the various types of RNA, there are many techniques for study of rRNA but PCR-DGGE technique is one of the most widely used which analysed 16S rRNA.

2.6.1 Polymerase chain reaction (PCR) technique

PCR is a powerful tool that allows the species-specific detection of organisms based on 16S rRNA amplification. It has been used not only for the

identification of isolated bacteria but also for analysis of food, clinical, and environmental samples. While it is easy to amplify DNA derived from pure cultures, problems arise if the sample investigated is as complex as food, soil, or biological waste, since the PCR is easily inhibited by numerous substances, including humic acids, fats, and proteins. Therefore, DNA has to be isolated and purified efficiently, and any PCR based procedure has to be critically evaluated for its detection limit and reliability. Furthermore, it has to be considered that when using PCR, the DNA of both viable and nonviable cells is amplified.

2.6.2 Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE was developed to detect changes in base sequence between DNA fragments. With the addition of GC-clamp to DNA fragment could be detected. This technique is the initial example of a series of gel melting techniques which allow sensitive and reproducible analysis of large numbers of samples. This ability to detect sequence has been exploited to combine the PCR of marker genes, especially 16S rDNA or rRNA, using conserved primers with the ability detect unknown polymorphisms amoung the mixed population of DNA fragments amplified from complex mixtures of these marker gene. The marker gene represents the species composition of an environmental sample.

Denaturing gradient gel electrophoresis of 16S rDNA fragment generated by the polymerase chain reaction (PCR-DGGE), has became popular method among microbial ecologist direct extraction of the community DNA and amplification of typically 200-600 bp long 16s rDNA fragments. These fragments are separated according to their melting point on a denaturing gel. The method also provides a comparison of the true sequences if DGGE bands are excised and sequenced. The PCR-DGGE was originally developed to analyse fragments from single organisms where comprehensive knowledge of the nucleotide sequence was available. Natural samples, however, typically contain a number of species with unknown phylogenetic affiliation and abundance. A relatively lower sensitivity of PCR-DGGE, when applied to natural samples and ecological question can therefore be anticipated, due to the lack of accurate knowledge of the nucleotide sequence obtained and the diversity of

natural bacteria communities. In correspondence with this low resolution in DGGE profiles has been reported when there is high diversity of bacteria in the sample.

2.7 Literature reviews

Lu et al.(1998) studied anaerobic degradation and toxicity of formaldehyde in batch culture with real wastewater from wood - glue wastewater and synthesis wastewater. The wood – glue wastewater had COD and formaldehyde in the ranges of 53,600 – 227,000 mg/l and 2,009 – 4,012 mg/l. The synthetic wastewater contained 5.5 g/l of glucose, mineral and trace element equivalent to COD and formaldehyde in the range of 6,675 – 40,500 mg/l and 0 – 3,000 mg/l. Both wastewaters were diluted with medium containing mineral and trace element for formaldehyde concentrations of 100, 200, 400 and 600 mg/l, respectively. The mix of sludge from wood processing wastewater treatment plant and domestic treatment plant were used during the start up period.

It was found that COD and formaldehyde removal efficiency were 70% and 90% from synthetic wastewater having formaldehyde concentration less than 400 mg/l and 92.4% and 98% from real wastewater having formaldehyde concentration less than 200 mg/l. Formaldehyde toxicity was found to depend on formaldehyde concentration, type of substrate and test time. The 50% inhibition of gas production was detected at formaldehyde concentration of about 300 mg/l. It was observed, when the formaldehyde concentration was at 400 mg/l for synthetic wastewater and 200 mg/l for real wastewater, that over 90% of the formaldehyde could be degraded. The anaerobic inhibition changed with the test time. The inhibition was 97% in the first 5 days. After 6 days, the inhibition decreased to 58% and no inhibition was observed after 7 days of experiment. The experiment showed that the system treating wastewater containing suitable substrate could tolerate higher formaldehyde concentration. Moreover, higher formaldehyde concentrations could be applied to the system when the microorganisms were given longer time to acclimatise.

Moteleb et al.(2002) studied the formaldehyde loading in an anaerobic granular activated carbon fluidized bed reactor treating high strength organic wastewater containing formaldehyde from a resin production facility. Nutrient and carbonate buffer solutions were added to sustain the biological growth and maintain

pH. The bioreactor was operated for 700 days with 2 parts of the experiment. In the first part, the reactor was continuously operated under 4 different organic loading rates: 0.1, 2, 4 and 2.5 kgDOC/m^3.d with formaldehyde concentrations of 0.035, 0.8, 2 and 1 kg HCOH/m^3.d, until steady state to find the proper hydraulic retention time. In the second part, effect of substrate perturbation on effluent quality was examined by periodically loading the reactor using five distinct perturbation to monitoring the shifts of production.

In the first part, the reactor removed 95% and 99.99% of the DOC and formaldehyde. The volatile fatty acids occurred from apply different organic loading rates did not inhibit the formaldehyde degradation. The removal mechanism was due to biological degradation and granular activated carbon adsorption. The limitation of the feed waste in 9 days of operation did not effect the degradation rates. It was claimed that formaldehyde was adsorbed by granular activated carbon. In the second part, the perturbation of the feed substrate did not have the impact on the treatment efficiency. Effluent concentrations of volatile fatty acids did not show the inhibition by formaldehyde under the conditions practiced in this study. The results of this study indicated that the formaldehyde degrading microorganism can be resilient when applying higher organic loading rate and perturbation by adding more FA concentration. Moreover, the granular activated carbon, used as a media, had ability in adsorbing formaldehyde in the system.

Oliveira et al. (2004) investigated the degradation and toxicity of formaldehyde in a Horizontal-Flow Anaerobic Immobilized Sludge Reactor. The reactor, 1000 mm long, 50.4 mm diameter, use polyurethane foam cubes as the media. The reactor was operated in temperature controlled chamber. The synthetic wastewater was prepared with COD and formaldehyde concentrations in the range of 51.6 – 1,798 mg/l and 26.2 - 1158.6mg /l. Before starting the experiments, the anaerobic reactor was used in treating phenolic synthetic wastewater with concentrations 50 - 1200 mg/l for 1 year.

The startup period was only 20 days. This rapid acclimatisation period was attributed to the previous reactor operation with phenol synthetic wastewater and to the high biomass retention provided by the polyurethane foam. The COD and formaldehyde removal efficiencies were 92% and 99.7%, respectively. The effluent

formaldehyde concentration showed slight variations as the influent concentration was increased. The accumulations of volatile fatty acids were not presented in the system because the biomass completely degraded these compounds to methane gas. Moreover, the formaldehyde degradation was completed by using a hydraulic retention time of 4.8 h which significantly less than those reported in other studies. The microorganism could adapt to substances and the products in the reactor, therefore the toxicity and inhibition problems were not occurred.

Raja et al.(2009) studied the treatment of formaldehyde containing wastewater in UAFB reactor with dimension 50.4 mm long, 33.5 mm diameter and used chemically inert insulated bead high 22.5 mm as the media. The empty bed volume and the void volume of the reactor were 760 and 360 mL, respectively. The reactor was fed with the synthetic wastewater at organic loading rate of 0.18–3.61 kg COD/m^3.d (COD concentration 100 – 2,000 mg/l) corresponding to formaldehyde concentration of 65–92mg/l at 14h HRT. In addition, nitrogen and phosphorus, and di-ammonium hydrogen phosphate (DAP) were also provided as nutrients after pH adjustment to 7 to sustain biological growth.

The COD and formaldehyde removal efficiencies were 24 - 92% and 41 - 99%, respectively. The efficiency of COD removal was decreased from 92% to 24% when increased organic loading rate from 0.18 kg COD/m^3.d to 3.61 kg COD/m^3.d . At the same time, the efficiency of formaldehyde removal was decreased from 99% to 41% when increased formaldehyde influences concentration from 65mg/l to 92 mg/l. Degradation of formaldehyde and COD decreased with decrease in HRT. When HRT was decreased from 24 to 6 h the formaldehyde, COD removal decreased from 99% to 83% and 91 to 31%, respectively. The formaldehyde and COD degradations efficiency were achieved from 90% to 99% when applying HRT at 14 h for the low COD concentrations (100 to 1000 mg/l). After that, increase of COD concentration up to 2000 mg/l leaded to decrease in removal efficiency to 41% due to its toxic effect. It can be concluded from this study that, UAFB reactor was suitable for the treatment of low strength formaldehyde containing wastewater.

Pereira et al. (2009) studied the degradation of formaldehyde in anaerobic sequencing batch biofilm reactor (ASBBR) in the lab - scale reactor. The polyurethane foam matrices were use as biomass immobilized. The reactor was fed

with the synthetic wastewater containing formaldehyde concentration of 31.6 - 1,104.4 mg/l at 35 ^{o}C with 8 h sequential for 212 days.

The formaldehyde removal efficiency was over 99%, with average effluent formaldehyde concentration of 3.6 ± 1.7 mg/L. When formaldehyde concentration was increased from 31.6 to 1,104 mg/l, the accumulations of non-degraded organic acids were observed in the effluent resulting in the COD removal efficiency being decreased (the present of organic matter in effluent COD values above 500 mg/l). As the result, the products from the formaldehyde degrading microorganisms had toxic effect to methane formation microorganism. Results from this work indicated that the formaldehyde degradation was more suitable in continuous-flow reactors with flow pattern close to plug flow than the CSTR because the specific biomass could grow along the reactor's length and adapt to specific compounds or products in the reactor (Oliveira et al., 2004). In CSTR, all of microorganism in the reactor contacted with toxic substances leading to the inhibition and accumulation of products.

Bolanos et al.(2001) studied phenol degradation in horizontal – flow anaerobic immobilized biomass reactor under mesophilic conditions. The reactor was made from bore – silicate tube with 1000 mm long, 50.4 mm diameter and used polyurethane foam cubes as the media. The reactor was operated for 8 months under temperature of 30 ^{o}C at hydraulic detention time 12 h. Phenol as the sole carbon and energy source was added under step – increased concentration from 50 to 1,200 mg/l. Trace metals, solution of salts and vitamins, were added as nutrient.

The start-up period was 33 days with phenol concentration 50 mg/l. The reactor fed with influent COD concentration of 1,028 mg/l achieved 98% and 99% COD and phenol removal efficiency. Moreover, the reactors successfully degraded higher concentration of phenol at 100, 300, 600, 900 and 1,200 mg/l after 148, 58, 47, 29 and 7 days, respectively. The result indicated that phenol degradation at very high concentrations could be succeeded in the reactor containing adapted microorganism.

Fang et al.(2004) studied anaerobic treatment of phenol in synthetic wastewater under thermophilic condition in UASB reactor. The synthetic wastewater contains 630 mg/l of phenol, corresponding to 1500 mg/l of COD and organic loading rate of 0.9 g-COD/l.d. The reactor operated under temperature of 55 ^{o}C with hydraulic retention times of 60, 48, 40 and 28 h for 224 days. In the startup period, the UASB

reactor was fed with phenol and sucrose as co – substrate. After steady state, this reactor was fed with only phenol as the sole carbon source.

The phenol removal efficiency was 99% at hydraulic retention time of 40 h. When HRT was lowered to 28 h, the removal efficiency dropped to 77%. This indicated that the bioactivity was inhibited by the increased phenol – loading rate at low HRT. However, the accumulation of volatile fatty acid was not observed in the reactor though out the operation period, indicating that UAFB reactor was suitable for the treatment of phenol containing wastewater.

The effects of the concentration of phenolic compounds on the biogas production and biodegradability were investigated by Hernandez et al. (2008). Seven phenolic compounds including phenol at concentration ranging 100 - 800 mg/l were study in this experiment. The inoculum used was collected from the anaerobic wastewater treatment plant in the resin production industrial. The phenolic compounds and the inculum were transferred to 1 l plastic bottle with aluminum cap to study the toxicity and biodegradability.

The result showed that phenolic compounds could inhibit the degradation of readily biodegradable organic fractions and their own biodegradation. In this work, assays were carried out under anaerobic conditions to study the inhibition of both gas production and biodegradability. An initial enhancement followed by an inhibition of biogas formation was found. The inhibition by the phenolic compounds was found to be influenced by autoxidation, apolarity, type, size and number of substitutions. Biogas production was reported to be influenced by concentration rather than any pH change.

Though removal of both FA and phenol under anaerobic condition using different types of anaerobic reactor has been investigated, study of simultaneous removal of these two compounds using real domestic wastewater as the co-substrate is still lacking. In this current work, removal of FA and phenol, the main ingredients of embalming fluid, in anaerobic filter using real domestic wastewater as the co-substrate was investigated.

CHAPTER III

METERIALS AND METHODS

3.1 Wastewater and inoculum

Domestic wastewater and inoculum were collected from the equilisation tank and sludge digester of the wastewater treatment plant at Chiang Mai University (Figure 3.1). Each litre of domestic wastewater contained 202.4±22.5 mg of COD, 88.0±10.4 mg of VSS, 157.7±18.3 mg of alkalinity and 54.3±19.9 mg of VFAs with the pH ranging from 6.8-7.0. The concentrations of MLSS and MLVSS of the sludge were 5985 and 4090 mg/l, respectively. This treatment plant receives in average 8,000 m^3/day of wastewater generated from Maharaj hospital and Chiang Mai University Campus.

Figure 3.1 Domestic wastewater treatment plant at Chiang Mai University

3.2 Embalming fluid

Embalming fluid was synthesised using analytical grade FA (J.T. Baker, USA) and phenol (Panreac, Spain) at the ratio of deionised water : FA : phenol of 100 : 4 :1 by volume. At this ratio, concentrations of FA and phenol were 35,322 mg/l and 5,280 mg/l, respectively. The synthesised embalming fluid was found to be acidic with the pH of 5, and therefore contained no alkalinity. The properties of FA and phenol were summarised in the Table 3.1.

Table 3.1 Chemical properties of FA and phenol

Properties	FA	Phenol
Molecular formula	CHOH	C_6H_5OH
Molar mass	30.02 g/mol	94.11 g/mol
Density	1.09 g/cm^3	1.0576 g/cm^3
Flashing point	56 °C	79 °C
Melting point	-92 °C	40.9 °C
Boiling point	-21 °C	182 °C
Solubility in water	Very high	8.3 g/100 ml (20 °C)
Acidity	-	pKa 10

*(ATSDR., 1989; Udomsinroj, 2003)

3.3 Anaerobic filter's media

Several supporting medias have been used in the anaerobic filter for treating FA and phenol (Vidal et al., 1999; Moteleb et al., 2002; Oliveira et al., 2004; Pereira and Zaiat, 2009). In this current study, plastic bioball (Figure 3.2), with the diameter of 4 cm and 90% void ratio, was utilised. Bioball was chosen because of its durability and high specific surface area (0.01137 m^2/m^3, measured using the Solid Work program).

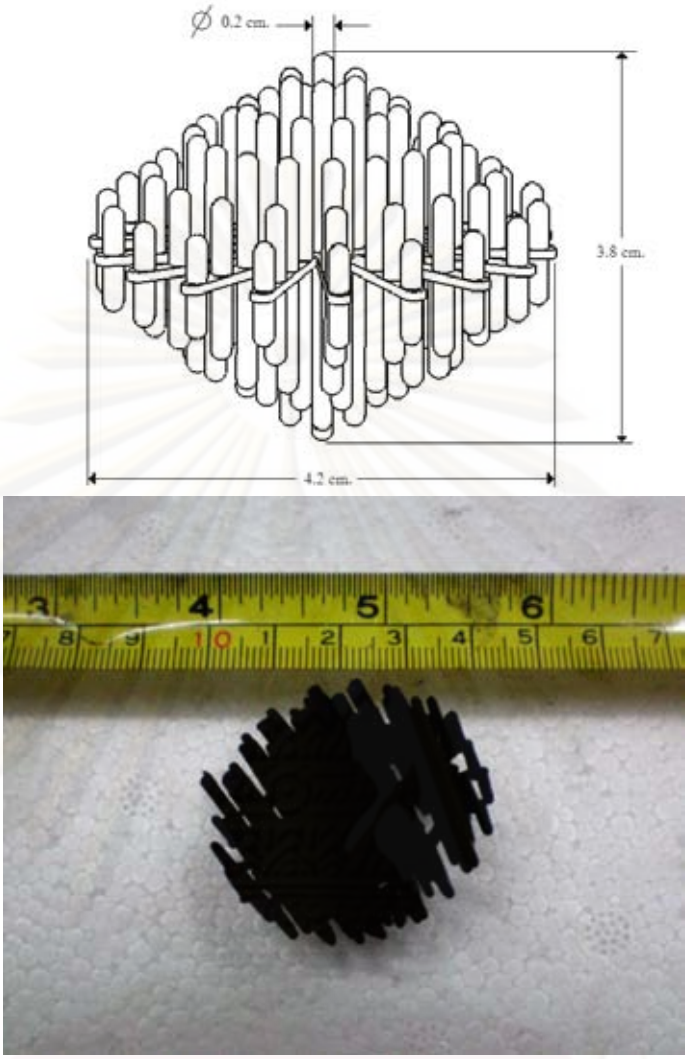


Figure 3.2 Dimensions of bioball

3.4 Lab-scale anaerobic filter

All experiments were conducted using two identical lab-scale upflow anaerobic filters (Figure 3.3). The reactor was constructed from PVC with a total volume of 10.2 L (10-cm diameter and 160-cm long). Of the 10.2 l total volume, the upper 7.1 l was the volume of packing media and the lower 3.1l under the media bed was allocated for suspended cultures (depth without media is 30% of the total working depth (Chernicharo, 2007)). Four sampling ports were installed at 30 cm, 65 cm, 90 cm, and 115 cm from the reactor's bottom and designated as ports a, b, c, and d, respectively. These ports were used for both water and sludge sampling. Biogas was collected and measured by water displacement method from the gas port on the top of the reactor in the 4 L plastic container. To make sure that all produced biogas

was measured, pH of water in the gas measurement container was adjusted to 6.8 using sulfuric acid solution to prevent CO_2 from being dissolved into the water.

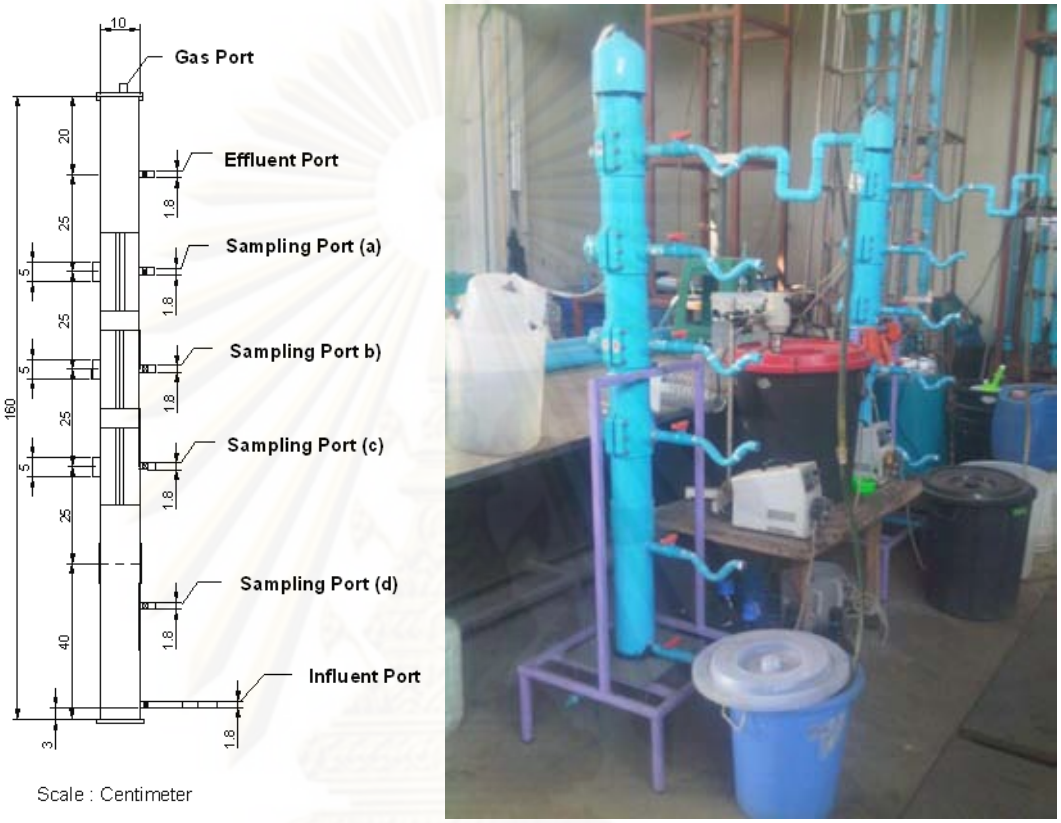


Figure 3.3 Lab-scale anaerobic filter

Figure 3.4 Biogas collection in the 4 L plastic container

3.5 Reactor operation and experimental setup

Before start up, 550 bioballs were randomly added in each reactor as the media. To start up the reactors, 7 L of inoculum was added and the domestic wastewater was fed into each reactor in the upflow mode at the desired flow rate of 40 and 20 l/d which corresponded to HRT of 6 and 12 hr (designated as Reactor A and Reactor B) until the steady state was reached (considering from stability of COD removal efficiency RSD<10%). After the completion of start-up period, embalming fluid was spiked into domestic wastewater starting at FA concentration of 22 mg/L and increased stepwise to the concentration 22, 64, 128, 410, 1374 mg/l, and 400 mg/l respectively. At each FA concentration, both reactors were operated until the steady state was reached. Effluent samples from both reactors were regularly collected at least 5 times after the steady state to determine the reactor performance. In addition, water and suspended sludge samples were collected along the reactor height at the end of each experiment to record the efficiency profile. The details of the experiments are presented in the Table 3.2.

Table 3.2 Detail of the experiment.

Experiment.	Embalming fluid: domestic wastewater ratio (V/V)	Concentration (mg/l)			Loading Rate (kg/m^3.d)						Experimental period (d)
					Reactor A(6h)			Reactor B (12h)			
		FA	Phenol	COD	FA	Phenol	COD	FA	Phenol	COD	
Start up	0 : 1			207			0.82			0.41	28
1	0.0007 : 1	22	5.4	204	0.09	0.02	0.82	0.05	0.01	0.41	32
2	0.002 : 1	64	15	394	0.25	0.06	1.57	0.18	0.03	0.79	23
3	0.004 : 1	128	33	477	0.51	0.14	1.91	0.26	0.07	0.96	25
4	0.013 : 1	410	82	844	1.64	0.32	3.38	0.82	0.16	1.69	27
5	0.040 : 1	1373	208	1756	5.49	0.84	7.02	2.80	0.42	3.56	35
6	0.013 : 1	400	78	751	1.60	0.32	3.00	0.80	0.16	1.50	45

*Reactor A and B were operated at 6h and 12h-HRT, respectively.

3.6 Physical and chemical analysis

3.6.1 Wastewater sampling and analysis

Samples were taken for measurement during each experiment. Details of sampling point, sampling frequency, and analytical method used are tabulated in Table 3.3.

Table 3.3 Wastewater sampling and analysis

Parameter	Inlet	Outlet	Frequency	Sample along the reactor depth	Frequency	Analytical Method
TCOD	√		2 times per week			Dichromate Reflux
FCOD		√	2 times per week	√	3 times during steady state	Dichromate Reflux
SS	√	√	2 times per week	√	Once a week	Gravimetric method
VSS	√	√	2 times per week	√	Once a week	Gravimetric method
Alkalinity	√	√	2 times per week	√	3 times during steady state	Titration method
Total VFA	√	√	2 times per week			
Specific VFA	√	√	Once a week	√	3 times during steady state	Gas Chromatograph – Flame ionization detector
pH	√	√	Every day			pH meter
Temperature	√	√	Every day			Thermo meter
FA	√	√	2 times per week	√	3 times during steady state	Direct Photometric Method
Phenol	√	√	2 times per week	√	3 times during steady state	Direct Photometric Method

Analysis of COD, alkalinity and solids was performed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1985). VFAs were measured using a Gas Chromatography (Hewlett-Packard, 1988).

3.6.2 VFAs analysis

Gas chromatography was used to analysis VFAs. Gas chromatograph utilized was equipped with a flame ionisation detector and HP-FFAP column (25m x 0.32mm x 0.5 μm). The injector temperature was 260˚C, with a split ratio of 1:10, and the detector temperature was 220 ˚C. The oven temperature was 80 ˚C for 1 min to120 ˚C at 120 ˚C/min and 170 ˚C for 6 min at 6 ˚C/min . H_2 was used as the carrier gas.

3.6.3 FA analysis

The concentrations of FA were measured according to standard method for Formaldehyde in water (ASTM, 2003).

3.6.3.1 Preparation of calibration curve of FA

To prepare FA solutions for the calibration curve, the FA standard concentration of 10 mg/l was diluted to 6 different concentrations (Table 3.4)

Table 3.4 FA solution preparation

no.	FA concentration(mg/l)	10 mg/l FA std. volume (ml)	DI water (ml)
1	0	0	6
2	0.5	0.3	5.7
3	1.0	0.6	5.4
4	2.5	1.5	4.5
5	5	3.0	3.0
6	7.5	4.5	1.5

Colour was developed after dilution. The absorbance was measured as described in Topic 3.6.3.2. A calibration curve was prepared by plotting the corrected absorbance (y axis) versus micrograms of FA (x axis). The linear equation was determined for the sample concentration calculation.

3.6.3.2 Sample measurement

Prior analysed, the sample was filtered through glass microfiber filter paper GF/C diameter 47 mm (Whatman). The filtered sample (6 ml) was then transferred with 6 ml of acyetyl acetone to a 40 ml glass vial tube with the cap and mixed for 30 min. In the case of turbid sample, 4 ml of n-butanol was added to eliminate the turbidity before mixing. Then, the glass vial tube was incubated at 60 C˚in the water bath for 10 min. After the incubation, the sample was transferred to the cuvette and measured the absorbence at the wave length of 412 nm. The absorbance of the FA from that of the standards and samples were compared to the calibration curve which obtained from Topic 3.6.3.1.

3.6.4 Phenol analysis

The colorimetric method was used to determine phenol concentration (APHA, 1985).

3.6.4.1 Preparation of calibration curve of phenol

To prepare the stock 100 mg/l phenol solution, 10 mg of phenol was dissolved in deionisation water. This stock phenol solutions were used further to prepare the calibration curve of phenol as shown in the Table 3.5.

Table 3.5 Phenol solution preparation

no.	Phenol concentration (mg/l)	100 mg/l phenol solution (ml)	DI water (ml)
1	0	0	100
2	1	1	99
3	2	2	98
4	3	3	97
5	4	4	96
6	5	5	95

Colour was developed after dilution. The absorbance was measured as described in Topic 3.6.4.2. A calibration curve was prepared by plotting the corrected

absorbance (y axis) versus micrograms of phenol (x axis). The linear equation was determined for the sample concentration calculation.

3.6.4.2 Sample measurement

The sample was filtered through glass microfiber filter paper GF/C diameter 47 mm (Whatman). The 100 ml of the sample and 2.5 ml of 0.5N ammonium hydroxide were then added in a 250 ml flask. The phosphate buffer (pH 6.8) was added immediately to adjust the pH to 7.9. After that 1 ml of 4-aminoantipyrine solution was, added in the flask and shaken for 10 sec. Potassium ferric cyanide solution (1 ml) was added to form the red colour. After 15 min, the sample was transformed to the cuvette and measured the absorbent at the wave length of 500 nm. The absorbance of the phenol from that of the standards and samples were compared to the calibration curve which obtained from Topic 3.6.4.1.

3.6.5 Biogas composition analysis

Biogas sample was taken directly from the gas measurement container using the biogas bag (Figure 3.5 Biogas sampling). Biogas composition was analysed by gas chromatography. The injector, oven and detector temperatures were 50 ˚C, 50 ˚C and 70 ˚C, respectively.

Figure 3.5 Biogas sampling

3.7 Microbiological observations and analysis

3.7.1 Characterisation of biomass

The biomass samples were taken from Ports b, c, and d, for microscopic examination by phase-contrast microscopy using an Olympus BX50F microscope. Biomass samples for optical microscopy examination were diluted with distilled water and immediately examined.

3.7.2 Sample fixation

To fix the sample, the MLVSS was taken from Ports b, c, and d and transferred to the 50 ml sterile plastic vial tube. The absolute ethanol was added at the ratio of 1:1 (by volume) to preserve the sample before stored at -20 °c. However, it was found later that the DNA could not be extracted from all samples fixed using this method. The reason for this problem was not clear but it was most likely to be caused by the reaction(s) between ethanol and some compounds present in the sample. This assumption was later supported when the DNA could be extracted from samples without the absolute ethanol. Several modifications of DNA extraction method were tried in the attempt to extract the DNA from the ethanol-fixed samples, including using the extraction kit (Vivatis GF-1 soil sample DNA and Nucleospin® Extract II Kits), with no avail. Samples were, then, fixed by only stored at –20 ˚C before DNA extraction and only those collected during Experiment 6 (Table 3.1) were extracted for DNA and undergone the PCR-DGGE procedure.

3.7.3 DNA extraction

DNA extraction conducted in this study was modified from the CTAB method (Robert. Grifftihs, 2000). The details of the protocol are summarized below.

Prior to extraction, 1ml of the MLVSS was thawed and transferred in a clean 2 ml eppendorf tube. The sample was centrifuged at 10,000 rpm for 5 min then the liquid was discharged. To wash the centrifuged sample, 1 ml of distilled water was added and mixed by inverting for 30 sec before centrifuging again at 10,000 rpm for 5 min. The liquid was discharged and repeated the washing step. Next, 0.5 g of glass beads (150-212 μm diameter) and 600 μl of the mixture of 5 M NaCl, 10%

cetyltrimethylammonium bromide (CTAB) and 100 mM phosphate buffer (pH 8) were added and mixed by vertex at maximum speed 1 min for 2 times, between vortexing sample was placed on ice. After cooling, 600 µl of liquefied phenol-chloroform-isoamylalcohol (25:24:1) was added and vertexed 2 min for 2 times. The tube was then centrifuged at 13,000 rpm in the fridge (preferably at 4 ˚C), microcentrifuged for 15 min before the supernatant was transferred to new clean 2 ml eppendorf tube. This step was repeated 2 times. Then, 600 µl of chloroform-isoamylalcohol (24:1) was added and inverted by hand for 30 min and centrifuged at 13,000 rpm for 15 min. The supernatant was transferred to new clean 2 ml eppendorf tube and filled with cool isopropanol and sodium acetate. Then, DNA was precipitated in the -70 ˚C freezer for 1 hr. After precipitating, the tube was centrifuged at 10,000 rpm for 10 min then the liquid was drained out. The DNA was washed twice in 70% ethanol and washed once in absolute ethanol. The DNA was dried overnight in the room temperature and then 200 µl of Rnase-TE buffer was added to suspend the DNA and kept at -20^{o}C until used.

3.7.4 PCR amplification

The extracted DNA from the previous step was used as the template for PCR amplification. The primers used for amplification of 16S rDNA of Achaea and Bacteria are listed in Table 3.2. The reactions were performed in a final volume of 50 µl contain 2 µl of extracted DNA (1/100 dilution) and 48 µl of master mix (the ingredient of the master mix depended on type of selective microorganism).

Table 3.6 The primers used for amplification of 16S rDNA of Archaea and Bacteria in this study.

Primers	Target	Primer sequence
F46	Achaea	5' TTAAGCCATGCGAAGT 3'
R1100	Achaea	5' TCGGGTCTCG CTCGTTGACC 3'
F340GC	Achaea	5'CGCCCGCCGCGCGCGGCGGGCGGGGCGGGG GCACGGGGGGCCTACGGG GCTGCAGCCAG 3'
R519	Achaea	5'TTA CCG CGG CGTG CTG 3'
F341GC	Bacteria	5'CGCCCGCCGCGCCCCGCGCCCGTCCCGCGCC CCCGCCCGCCTACGGGAGGCAGCAG 3'
R534	Bacteria	5'ATTACCGCGGCTGCT GG-3'

For the archaea, the master mix for contained 2 µl of 0.01 mM stock forward primer, 2 µl of 0.01mM stock reverse primer, 2 µl of 5 mM dNTPs (dATP,dGTP, dTTP, dCTP), 6 µl of 50 mM $MgCl_2$, 5 µl Taq buffer buffer, 2 µl of DMSO, 0.6 µl Taq DNA Polymerase (5 u/ml) and 39.3 µl of sterile deionisation water. Amplification reactions for F46-R1100 primer were performed in a MJ Research PTC-200 thermal cycler, according to the following profile: 2 min at 92°C and 35 cycles of 1 min at 92°C, 30 sec at 55°C and 1 min at 72°C, followed by 6 min at 72°C. The next step were used the PCR results of F46-R1100 primer amplified by used the F340GC-R519 according to the follow profile: 4 min at 94°C and 35 cycles of 30 sec at 94°C, 1 min at 55°C and 1 min at 72°C, followed by 6 min at 72°C.

For bacteria, the master mix contained 2 µl of 0.01 mM stock forward primer, 2 µl of 0.01mM stock reverse primer, 2 µl of 5 mM dNTPs (dATP,dGTP, dTTP, dCTP), 6 µl of 50 mM $MgCl_2$, 5 µl Taq buffer buffer, 2 µl of DMSO, 0.6 µl Taq DNA Polymerase (5 u/ml) and 28.4 µl of sterile deionisation water. Amplification reactions according to the following profile: 3 min at 94°C and 35 cycles of 30 sec at 94°C, 1 min at 54°C and 1 min at 72°C, followed by 5 min at 72°C.

Amplification products were analyzed by electrophoresis in 1% (w/v) agarose gels stained with ethidium bromide. After the PCR was completed, the templates were stored at -20 ˚C.

3.7.5 Denaturing Gradient Gel Electrophoresis (DGGE)

The DGGE was performed using a Universal Mutation System (Bio-Rad, Hemel Hempstead, UK). The 8% polyacrylamide gel was composed of 0.1% (v/v) tetramethylenediamine (TEMED), 0.1% (v/v) ammonium persulfate, 50X TAE buffer and 40% 37.5:1 acrylamide/N, N'-methylenebisarcylamide solution. A gel was prepared with the denaturing gradient ranging from 35% to 60% for archaea and 20-60% for bacteria where 100% denaturant contains 7 M urea and 40% formamide deionised with AG501-X8 mixed-bed resin. Gels were allowed to polymerise overnight. Electrophoresis was conducted in a 1X TAE buffer for 3 h at 200 V and 60 ˚C.

3.8 The Statistical analysis

Statistical analysis was conducted using the Minitab program (Minitab, USA). Comparison of means of two samples was done using the 2 samples t-test at the confidence level of 95%.

CHAPTER IV

RESULTS AND DISCUSSION

As stated in Chapter 1, the objectives of this work were to; (1) study effects of organic loading rate and hydraulic retention time on the anaerobic filter performance during removal of formaldehyde and phenol, the main ingredients of embalming fluid, (2) determine the maximum amount of embalming fluid in domestic wastewater that can be treated by anaerobic filter (3) investigate the role of microbial communities growing in anaerobic filter operated at different conditions in degrading formaldehyde and phenol. Results and the corresponding discussion of each experiment done to fulfill each objective are presented in this chapter.

4.1 Physical and chemical analysis during start-up period

Two anaerobic filters were started up under different flow rates which corresponded to HRT of 6h (Reactor A) and 12h (Reactor B) until the steady state was reached (considering from the stability of COD removal efficiency). The sludge from a sludge digester of Chiang Mai University wastewater treatment plant was used as inoculum. Wastewater from the same source was chosen to shorten the start-up period.

Both reactors required only 12 d to reach the steady state. Profiles of temperature, pH, alkalinity, VFA, SS, VSS, COD, gas volume and gas composition were analysed by time to monitor the reactor performances.

4.1.1 Temperature

The anaerobic degradation is found to function well under mesophilic condition (20 -35 ˚C) (Speece, 1996). The temperature should be monitored because decrease in specific microorganisms activity and reactor efficiency may occur when temperature changes in the anaerobic filter. The measured temperature was in the range of 23 - 26˚C for both influent and effluent samples. These temperatures were

within the suitable range for anaerobic degradation (20 - 35˚C) implying that the anaerobic microorganisms inside both reactors were functioning at their optimal temperature.

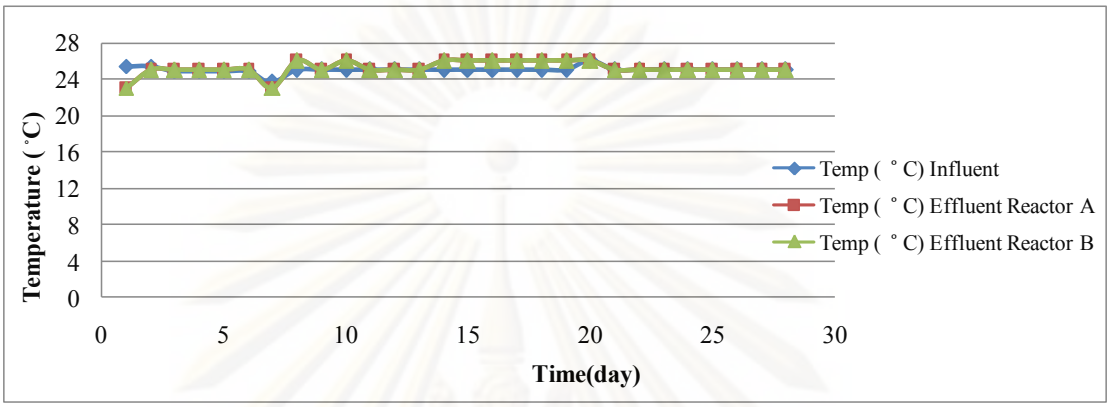


Figure 4.1 Temperature during the start up period.

Slight temperature variations were observed in influent and effluent of both reactors due to variations of ambient air temperature (Figure 4.1). This reason could also explain the higher temperatures of some effluent samples compared to those of the influent ones as influent and effluent sample were collected at the different time of a day.

4.1.2 pH

pH values can be used as an indicator for anaerobic reactor's stability. The methanogenic activity is more likely to proceed optimally in a narrow pH range, i.e. between 6.3 and 7.8 (Van Haandel A., 2007) compared to 5.5-6.3 for the acid formers. Results from this study (Figure 4.2) showed that the pH values were in the optimum ranges, 6.72-7.35 and 6.75-7.8, for the effluent samples of Reactors A and B, respectively.

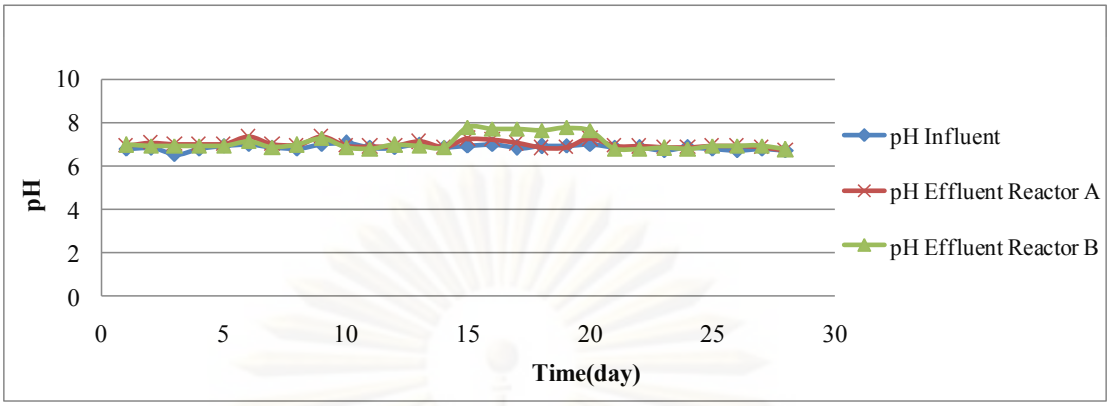


Figure 4.2 pH during the start up period

The suitable ranges of pH were maintained because the domestic wastewater used in this study contained sufficient buffer capacity as explained in Topic 4.1.3.

4.1.3 Alkalinity and Volatile Fatty Acid (VFA)

The alkalinity in wastewater helps to resist the change in pH. Domestic wastewater is normally alkaline, receiving its alkalinity concentrations measured from the materials added during domestic use. The alkalinity is normally present in the bicarbonate (HCO_3^-) form (Metcalf&Eddy, 2004). The alkalinity concentrations measured in this study were in the ranges of 140-200, 156-200 and 174-198 mg/l for influent and effluent samples of Reactors A and B, respectively (Figure 4.3).

The VFA is a decisive factor for the balance of acidogens and methanogens population. Moreover, the accumulation of VFA affected the performance of methanogens in producing methane. In the steady state, concentration of VFA should be low and considerably stable. The VFA concentration in the influent was in the range of 38-66 mg/l, while those of the effluents from Reactors A and B were in the range of 38-58 and 40-64 mg/l, respectively (Figure 4.4). The VFA concentration in day of 20th was higher than other prior day of the operation because of the higher amount of VFA concentration in the raw domestic wastewater.

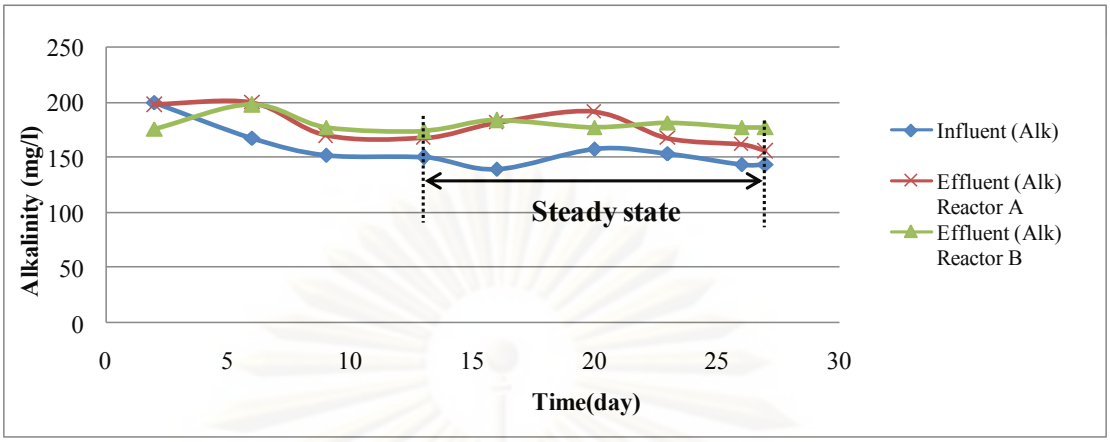


Figure 4.3 Alkalinity during the start up period

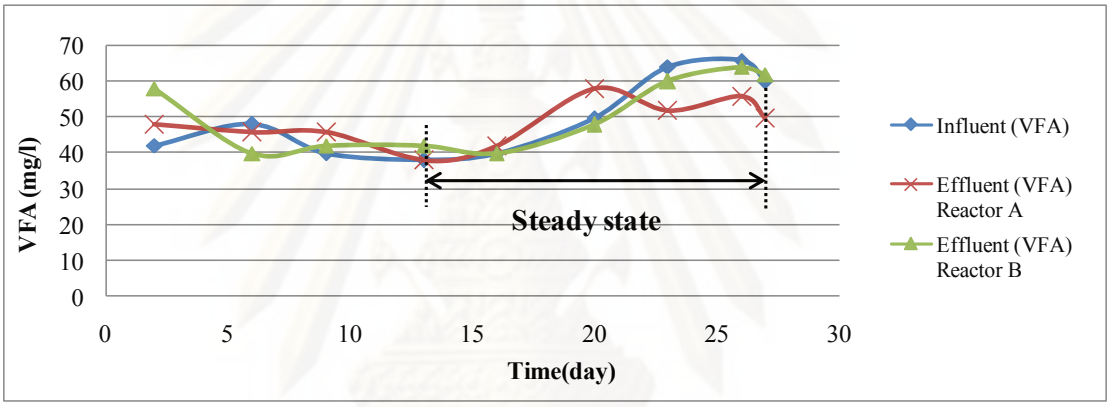


Figure 4.4 VFA during the start up period.

McCartry (1986) suggested that the suitable ratio of VFA to alkalinity for the anaerobic process activity should be less than 0.4. The ratio of VFA to alkalinity in the effluent of both Reactors was calculated to be within the suitable range (0.28 in both of Reactors A and B). Moreover, the alkalinity variation and VFA accumulation were not found during the start up period, indicating the stability of anaerobic process.

4.1.4 Suspended Solid (SS) and Volatile Suspended Solid (VSS)

Wastewater contains a variety of solid materials varying from rags to colloidal material. The fact that the distinction between colloidal particles and truly dissolved material has not been made routinely has led to confusion in the analysis of treatment plant performance. In general, SS and VSS are presumed to be organic material in the water. The difference is SS contain more complex material than VSS. Moreover, VSS concentration in the effluent sample could be determined as the

amounts of microorganism wash out. Therefore, the SS and VSS results are used routinely to assess the performance of the conventional treatment process.

4.1.4.1 Suspended Solid (SS)

The influent SS concentration during the start up period was in the range of 84-124 mg/l with the average value at the steady state of 104±3 mg/l. The average effluent SS concentrations were 17±5 and 9±5 mg/l for Reactors A and B, respectively (Figure 4.5). The calculated SS removal efficiencies, using values in the steady state, were 83±6 and 96±5 for the Reactor A and B, respectively (Figure 4.6).

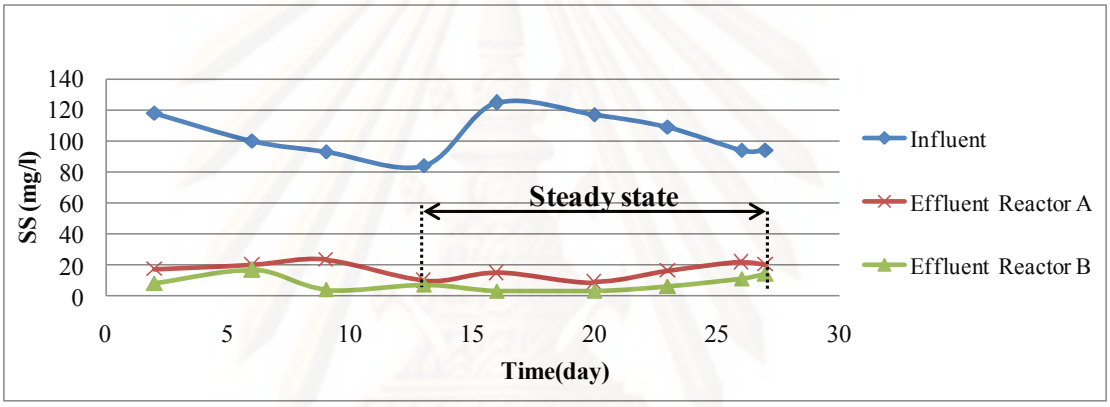


Figure 4.5 SS concentration during the start up period.

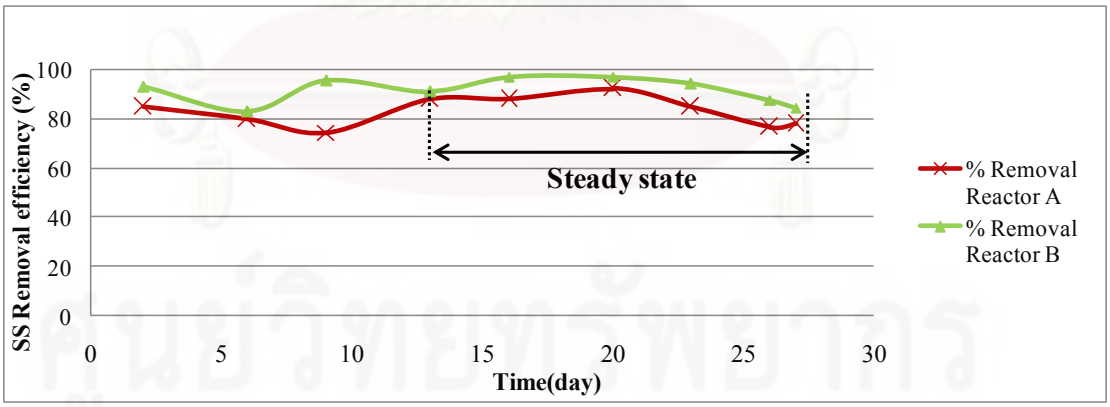


Figure 4.6 SS removal efficiency during the start up period.

Expectedly, at 95% confidence level using 2 samples t-test, the SS removal efficiency of Reactor B was significantly higher than that of Reactor A ($P = 0.005$, $P<0.05$). This was because at longer HRT, SS had longer time to settle leading to higher SS being removed.

4.1.4.2 Volatile Suspended Solid (VSS)

The influent VSS concentration was in the range of 69-109 mg/l. The average influent VSS concentration was 88±10 mg/l. While the average effluent VSS concentrations were 9±4 and 5±2 mg/l for Reactors A and B, respectively (Figure 4.7). The average VSS removal efficiencies for reactor during the steady state were 90±5 and 94±3 mg/l for the effluent of Reactors A and B, respectively (Figure 4.8).

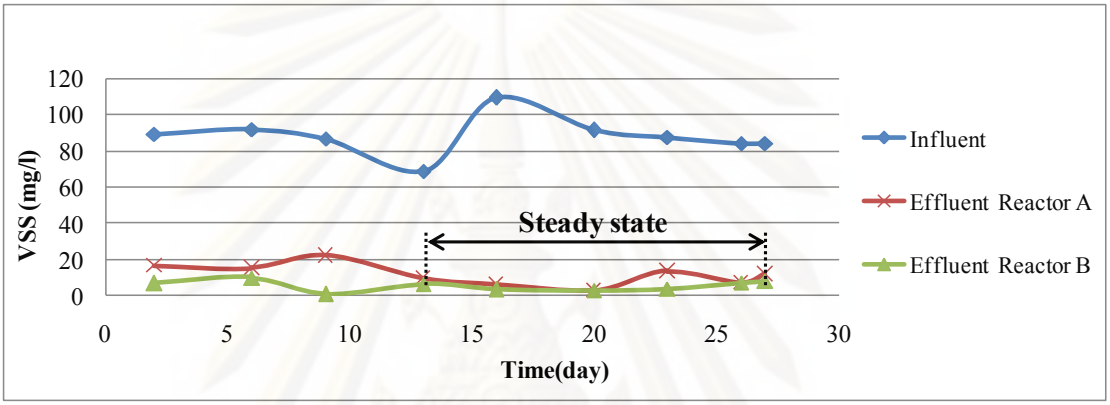


Figure 4.7 VSS concentration during the start up period

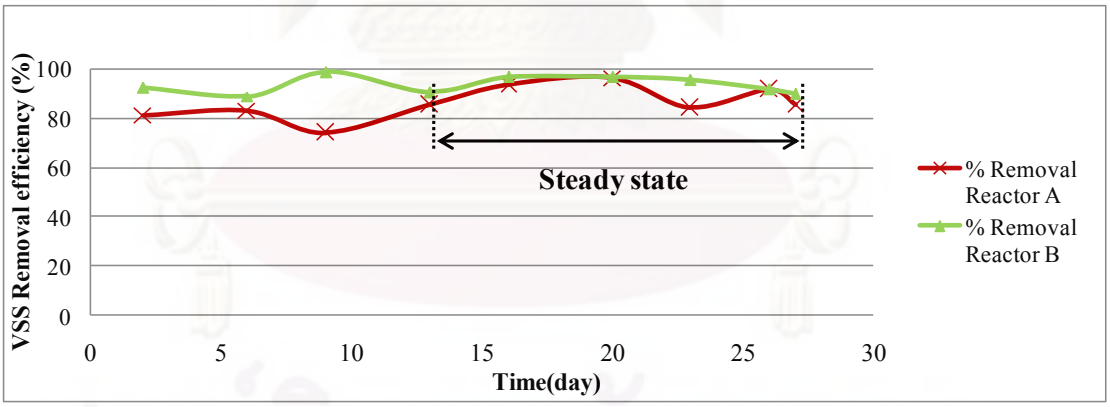


Figure 4.8 VSS removal efficiency during the start up period.

As the wastewater used in this study was taken from the equalization tank of the separate sewer system, low concentrations of SS and VSS were observed. Both SS and VSS concentrations measured in this study were similar to those reported by Metcalf&Eddy, (2004), for the typical concentrations of SS and VSS in the low strength domestic wastewater (120 and 95 mg/l, respectively). Although, the same trend of concentration and removal efficiency was observed for SS and VSS. The average ratio of VSS to SS in the influent sample was 83%, indicating that the

majority of suspended solids were organic substances. The 2 samples t-test analysis showed that VSS removal efficiency was higher in Reactor B than Reactor A (P = 0.011, P<0.05). Lower VSS removal efficiency found in the Reactor A might be the result of more biomass being washed out in the effluent from this reactor.

4.1.5 COD

The removal of organic substances by heterogeneous microorganisms in the anaerobic filters can be determined using the COD concentration and removal efficiency. The influent COD concentration was in the range of 169-235 mg/l. The average influent COD concentration at steady state was 202.4±22.6 mg/l (Figure 4.9). During this period, high COD removal efficiencies were detected in both reactors (79±5% and 85±3% in Reactors A and B, respectively; Figure 4.10).

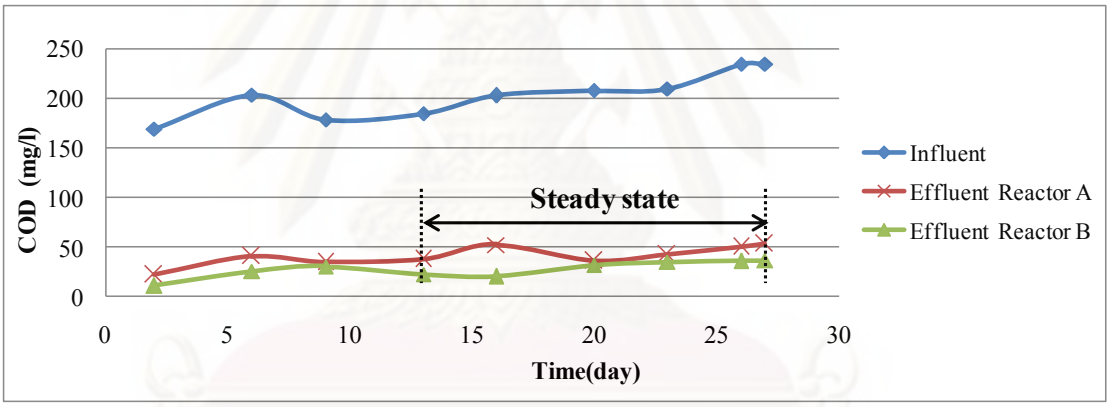


Figure 4.9 COD concentration during the start up period

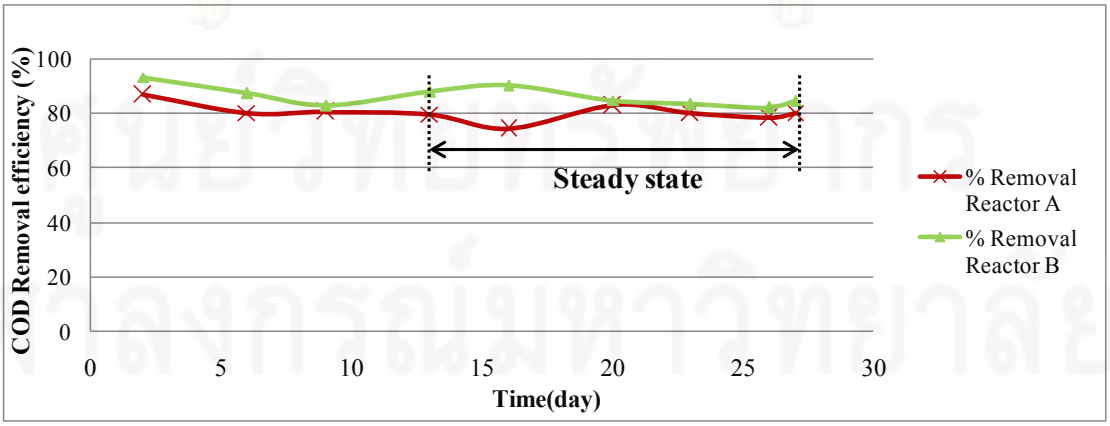


Figure 4.10 COD removal efficiency during the start up period

Slight variation of influent COD was found because of real wastewater was used. The influent COD concentration measured in this study was similar to the normal COD concentration in the low strength domestic wastewater reported in Metcalf&Eddy (2004). Using the 2 samples t-test, significantly (P = 0.017) higher COD removal efficiency was found in the Reactor B compared to that of the Reactor A. This result supported the benefit of operating the anaerobic filter under longer HRT in removing the organic matter (Speece, 1996).

4.1.6 Gas volume and gas composition

The principal end product of the anaerobic biodegradation of the organic matter is biogas. It normally contains about 65-70% CH_4 by volume, 25-30% CO_2, and small amount of N_2, H_2, H_2S, water vapor and other gases (Chernicharo, 2007). The amount of gas production depends on the balances in microbial population and microbial activity in the anaerobic filter. Stability of gas production and gas composition can be obtained, if balance condition between two main groups of microorganisms; the acid and methane formers, is maintained.

The average gas volumes during the steady state were 1.41±0.16 and 1.13±0.12 l/d for Reactors A and B, respectively (Figure 4.11). The CH_4 compositions in the biogas were 49±1 and 53±1% for Reactors A and B, while the CO_2 compositions were 43±1 and 42±2% for Reactors A and B, respectively (Figure 4.12).

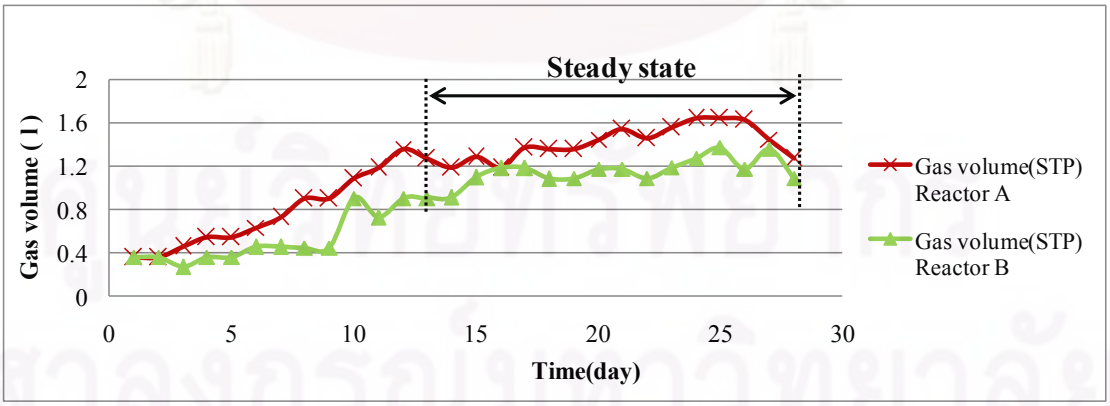


Figure 4.11 The volume of gas production during the star up period.

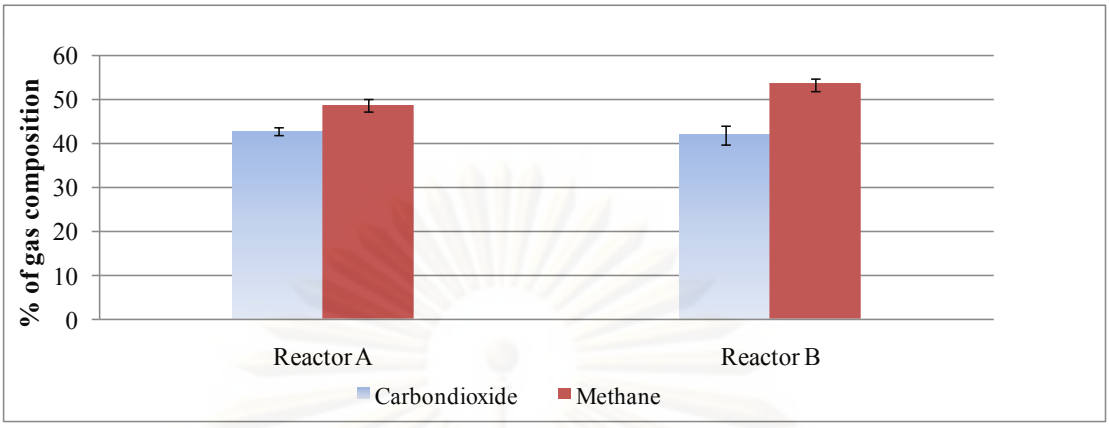


Figure 4.12 The composition of gas production during the start up period

Higher amount of biogas was produced in the Reactor A as this reactor was fed at higher organic loading rate. Regarding the gas composition, percentage of CH_4 in the biogas produced from both reactors was relatively lower than that normally found. This was the result of CO_2 composition in the biogas being high, which could be explained using the relationship of CO_2 partial pressure, pH, and alkalinity (Figure 4.13). As alkalinity of the effluents from both reactors was quite low (less than 200 mg/l) with the pH in the lower than 7.0 region, partial pressure of CO_2 in the biogas could be high, resulting in the lows CH_4 composition detected.

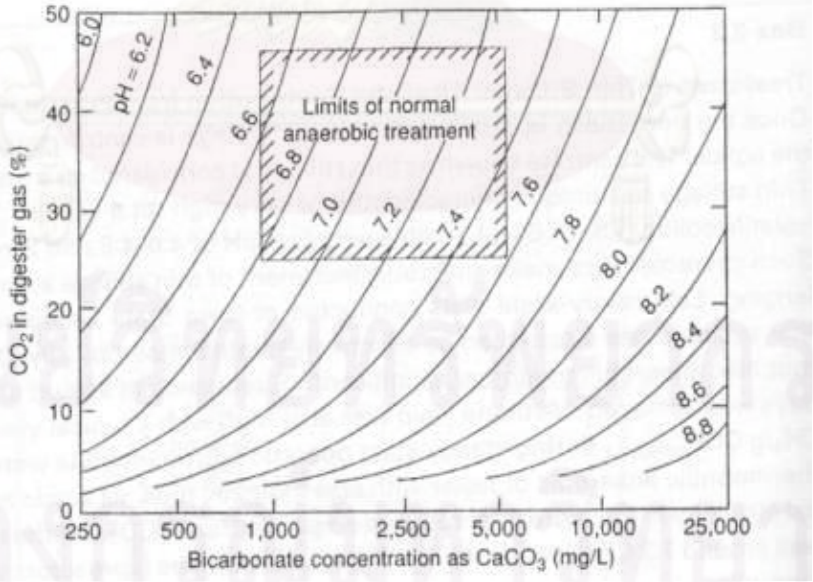


Figure 4.13 Partial pressure of CO_2 in biogas as a function of alkalinity for difference values of pH (Khanal, 2008).

The specific methane yield (lCH_4 / gCOD removed) is the parameter that characterises the volume of CH_4 production in relation to the organic matter removal from the biomass activity. The specific methane yields during the start-up period depend on type of seed sludge and its acclimatization to the wastewater being treated. The specific methane yields calculated (Figure 4.14) 0.10 and 0.17 LCH_4 / gCOD removed in Reactors A and B, being within the range of the recommended specific CH_4 yield during start-up, i.e. 0.10-0.50 lCH_4/gCOD removed (Chernicharo, 2007).

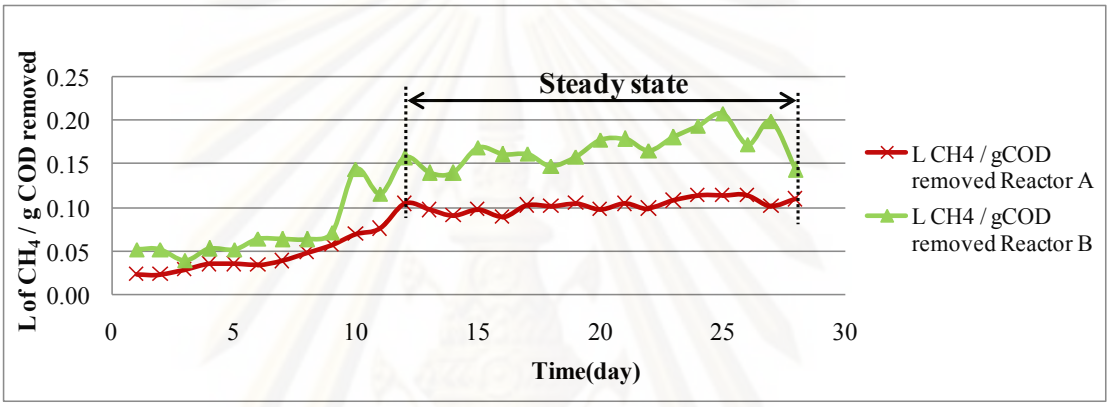


Figure 4.14 Specific methane yield (L of CH_4/ gCOD) during the start up period.

The specific methane yield of the Reactor B was found to be significantly higher than that of the Reactor A (P=0.000). This might be attributed to the fact that at longer HRT, microorganisms had longer time to degrade the organic carbon and therefore, gaining higher specific methane yield. This result was also supported by higher COD removal obtained in Reactor B (12h-HRT) as relatively higher portion of COD was utilized in methane production.

4.1.7 Profile of parameters along reactor's height.

Samples were collected from five sampling ports; influent Port, Port b, Port c, Port d and effluent ports to construct profiles of COD, SS and VSS. Results are tabulated in Table 4.1.

Table 4.1 The profile of parameters collected from Reactors A and B

Sampling port	Height (cm)	Reactor A				Reactor B			
		COD	SS	VSS	VSS/SS	COD	SS	VSS	VSS/SS
Effluent	0	237				237			
b	30	128	7700	5161	0.63	70	5620	3951	0.70
c	65	78	9555	6058	0.63	66	11153	6967	0.62
d	90	47	5971	3827	0.64	54	10444	6421	0.61
Influent	140	50				44			

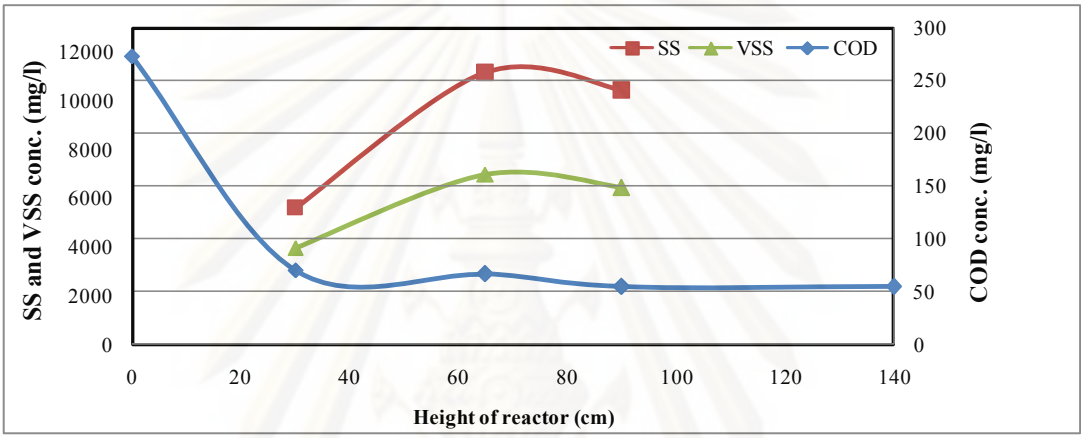


Figure 4.15 The Reactor A profile in the start up period

From the Reactor A's profile (Figure 4.15), COD concentration decreased along the reactor height to a stable value at Port c. COD concentration measured at this port was close to the effluent COD concentration. This result showed that the height longer than 90 cm did not contribute to further organic degradation. For the purpose of COD removal, therefore, anaerobic filter with the height 90 cm is enough to achieve maximum performance efficiency. On the other hand, the maximum concentrations of SS and VSS were observed in Port b. As reported by Tay (1999), the suspended sludge portion contributed the most to activities inside anaerobic filter. The VSS concentrations measured in the Reactor A showed that high amount of anaerobic sludge could be maintained in the studied reactor which should be beneficial to the reactor performance. Moreover, ratios of VSS to SS calculated (Table 4.1) were quite constant with the average of 0.63. This means that proportion of microorganisms to other inert solid was constant along the active height of Reactor And no accumulation of the non-sludge solids was occurred.

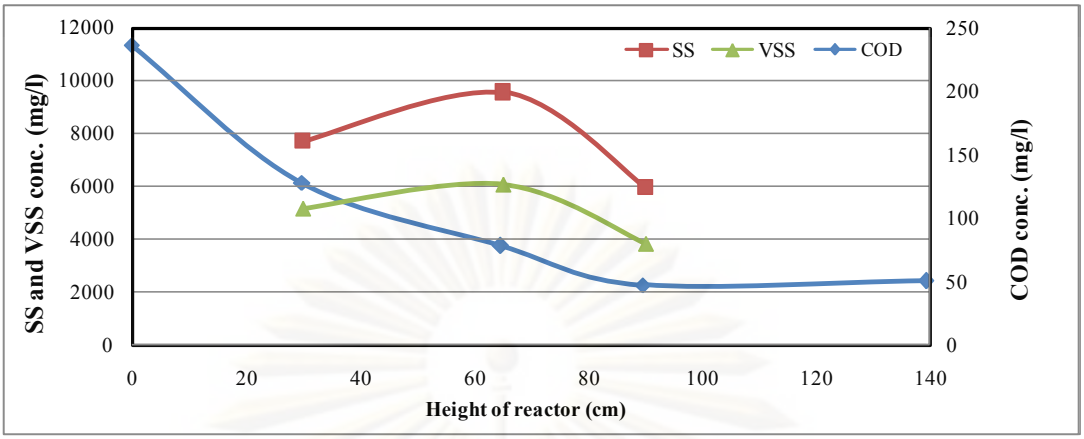


Figure 4.16 The Reactor B profile in the start up period

The Reactor B's profile (Figure 4.16) was somehow similar to that of Reactor A. The difference was the COD profile, in which that of Reactor B, the COD concentrations were suddenly reduced to the relative constant value at Port b rather than gradually reduced as in Reactor A. This sharp reduction of COD could be the results of longer HRT as microorganisms had longer time to transform the organic substances. The other possible reason was that there might be some differences between microbial communities existed inside Reactors A and B. According to Pholchan, M.K. et al., (2010), based on experimental results obtained from reactors operated with different organic loading rates, low organic loading rates increased the performance of the Reactor and the diversity of microorganisms. Though the VSS concentration at port a of the Reactor B was found to be relatively lower, the microorganisms might be more diverse and contributed to the better kinetic activity.

4.2 Reactor performance after embalming fluid addition.

After completion of start-up step, embalming fluid was spiked with domestic waster at the increasing step ranging from 22 to 1373 in form of FA concentrations. At 1373 mgFA/l corresponding to 208 mg phenol/l (Experiment 5), reactor performance was found to be dramatically deteriorated. The embalming fluid was, then spiked at the previous concentration (400 mgFA/l and 78 mg/l phenol ; Experiment 6) to monitor the recovery capability of the reactor performance. At each operational concentration, both reactors were operated until the steady state was

reached. The reactor performance achieved for every experiment is summarised in Table 4.2.

Table 4.2 Reactor performance obtained from every conducted experiment

Experiment no.	Time (day)	COD				
		Concentration (mg/l)			Efficiency (%)	
		influent	Reactor A	Reactor B	Reactor A	Reactor B
Start up	28	207±18	47±7	28±6	79±3	39±7
1	32	204±24	41±6	166±21	83±5	90±3
2	23	394±11	56±11	344±12	88±3	91±3
3	25	477±7	126±11	356±19	74±2	95±1
4	27	844±51	392±33	449±31	53±2	66±3
5	35	1756±73	810±19	928±30	54±1	64±4
6	45	751±15	334±74	396±39	55±8	77±13

*Reactor A and B were operated at 6h and 12h-HRT, respectively.

Table 4.2 Reactor performance obtained from every conduct experiment (cont.).

Experiment no.	Time (day)	SS					VSS				
		Concentration (mg/l)			Efficiency (%)		Concentration (mg/l)			Efficiency (%)	
		influent	Reactor A	Reactor B	Reactor A	Reactor B	influent	Reactor A	Reactor B	Reactor A	Reactor B
Start up	28	104±15.	16±5	8± 4	85±6	92±5	88±14	8±4	5±2	90±5	94±3
1	32	120±43	50±58	8± 5	73±25	86±18	73±40	9±6	6±2	92±4	91±3
2	23	38±6	23±6	7±3	41±7	77±7	30±11	5±3	4±2	83±8	86±5
3	25	47±8	19±6	20±8	72±17	74±14	46±11	18±12	17±13	77±16	88±9
4	27	47±13	28± 5	14±11	57±20	75±16	45±11	11±9	11±13	86±13	79±19
5	35	76±9	28±21	21±11	83±8	81±7	56±12	13±6	13±6	80±11	75±12
6	45	115±19	30±16	20±10	74±12	83±6	68±15	24±13	18±13	60±22	90±7

Table 4.2 Reactor performance obtained from every conduct experiment (cont.).

Experiment no.	Time (day)	FA					Phenol				
		Concentration (mg/l)			Efficiency (%)		Concentration (mg/l)			Efficiency (%)	
		influent	Reactor A	Reactor B	Reactor A	Reactor B	influent	Reactor A	Reactor B	Reactor A	Reactor B
Start up	28										
1	32	22±0.40	0.7±0.39	0.6±0.31	97.0±1.65	97.2±1.42	5.4±1.20	0.9±0.76	0.3±0.16	88±7.46	94±2.05
2	23	64±2.30	0.5±0.15	0.3±0.15	99.2± 0.44	99.3±0.30	15±1.42	5.0±0.79	0.1±0.06	67±2.63	99±0.47
3	25	128±6.78	13±0.49	1.2±0.06	99.6±0.14	99.7±0.15	33±1.69	22±1.36	0.6±0.13	34± 1.77	98±0.33
4	27	410±2.89	14±0.30	1.3±0.07	96.6±0.09	99.7±0.00	82±1.59	58±0.83	27±0.81	28±1.09	67±1.45
5	35	1373±32.80	1.6±0.21	1.6±0.21	99.0±0.00	99.9±0.00	208±12.36	190± 11.29	177±10.51	8.7±0.27	15±0.62
6	45	400±18.20	0.8±0.56	0.6±0.38	99.7±0.16	99.8±0.07	78±4.07	56±2.84	42±17.90	27±4.01	47±21.78

Table 4.2 Reactor performance obtained from eve conduct experiment (cont.).

Experiment no.	Time (day)	Gas						Methane Yield (LCH4/gCOD removed)	
		Gas volume (L)		CH_4(%)		CO_2(%)			
		Reactor A	Reactor B	Reactor A	Reactor B	Reactor A	Reactor B	Reactor A	Reactor B
Start up	28	1.4±0.08	1.1±0.05	47.97	53.43	42.733	41.9	0.10±0.006	0.17±0.019
1	32	0.5±0.10	0.4±0.13	54.35	56.15	44.15	33.55	0.05±0.000	0.07±0.012
2	23	1.2±0.05	1.1± 0.08	56.3	56.15	37.05	33.55	0.06±0.005	0.10±0.004
3	25	2.0±0.20	1.3±0.12	57.85	62.05	36.25	34.05	0.09±0.089	0.10±0.089
4	27	2.0±0.15	1.4±0.05	68.45	69.85	26.85	24.75	0.08±0.000	0.08±0.008
5	35	4.6±0.17	4.7±0.13	76.77	76.18	14.267	14.55	0.09±0.005	0.16±0.008
6	45	1.2±0.04	1.6±0.17	67.3	72.8	25.9	29.7	0.05±0.013	0.14±0.008

4.2.1 Temperature

The temperatures for the whole experimental period somewhat fluctuated in the range of 18 – 29°C (Figure 4.17). The temperatures of some effluent samples were slightly increased because of difference in ambient air temperature during the sampling times. However, the operating temperatures of this study were within the suitable range for anaerobic degradation (20-35°C).

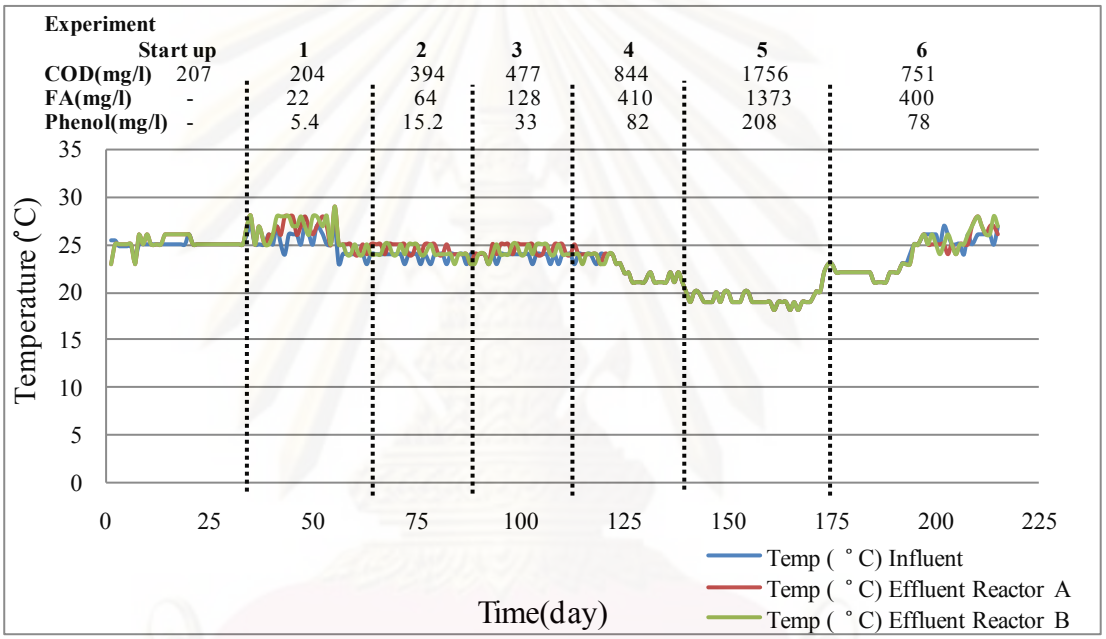


Figure 4.17 The temperature during the whole experimental period

At 20° (Experiment 5), anaerobic filters used in this study could still normally function, judging from the methane yield (Table 4.2), since the sufficient residence time for methane-producing bacteria was provided.

4.2.2 pH and alkalinity

The average values of pH and alkalinity in this study are shown in the Table 4.3, Figure 4.18 and Figure 4.19 show the pH and alkalinity throughout the whole experimental period.

Table 4.3 Influent and effluent pH and alkalinity observed in this study.

	pH			Alkalinity		
		Effluent			Effluent	
	Influent	Reactor A	Reactor B	Influent	Reactor A	Reactor B
Min.	7	7.38	7.8	134	124	116
Max.	6	6	6	292	272	288
Average	7.02	6.74	6.77	177	180	186
SD.	0.14	0.27	0.32	38	33	42

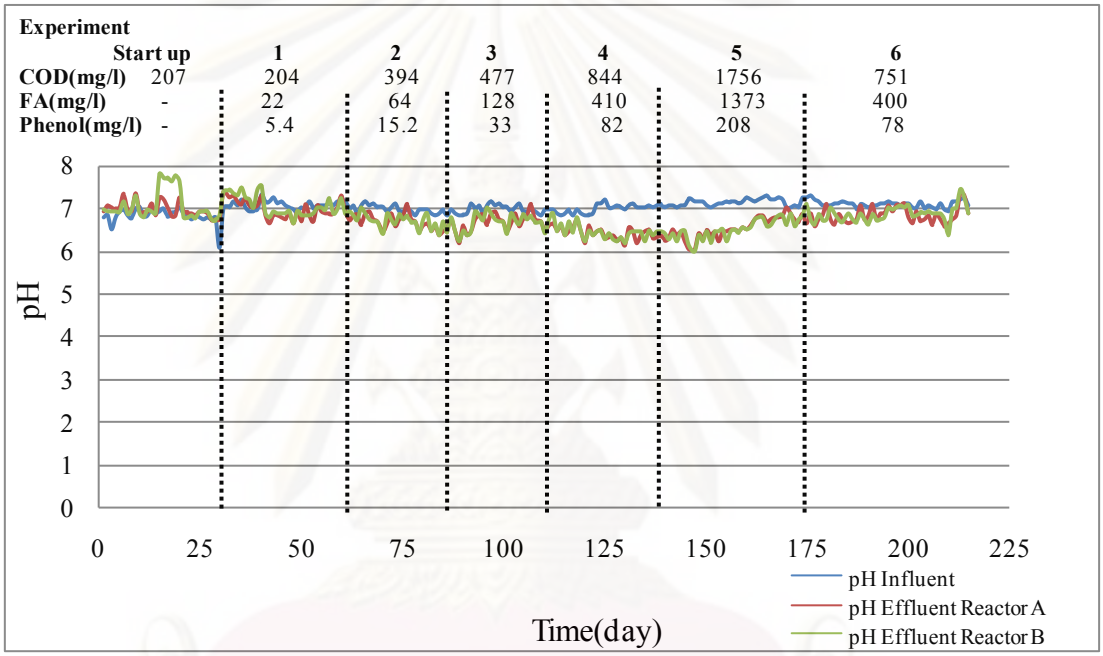


Figure 4.18 pH during the whole experimental period

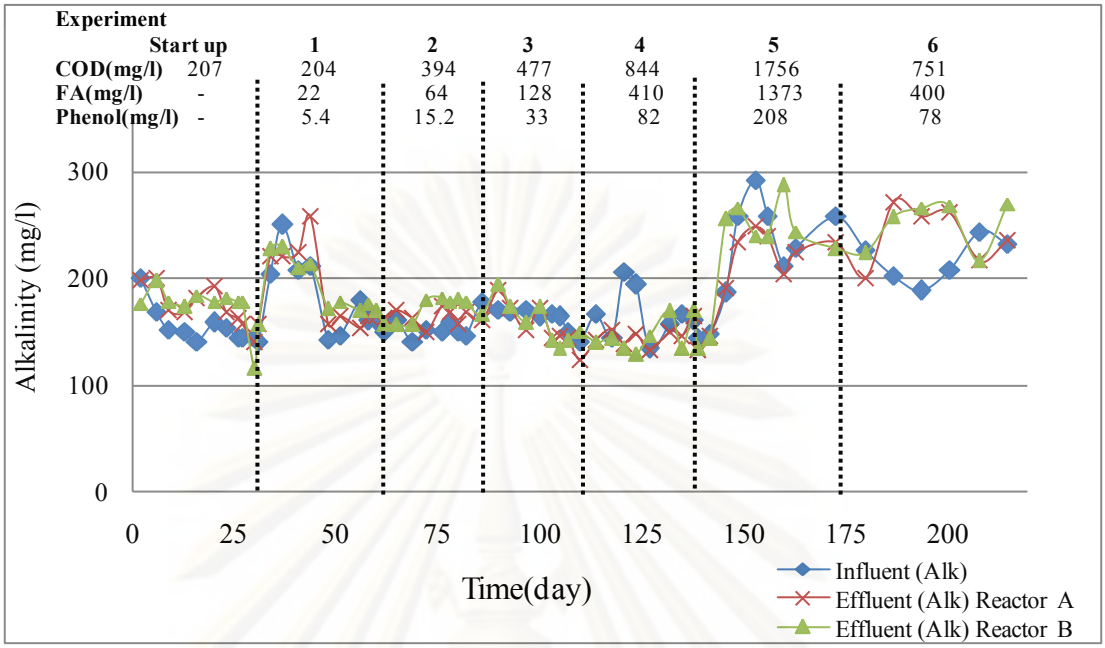


Figure 4.19 Alkalinity during the whole experimental period

pH in the influent sample was suddenly dropped to 6 when started feeding the embalming fluid. The value and stability of pH in an anaerobic Reactor Are extremely important because methanogenesis only proceeds at a high rate when the pH is maintained in the natural range (Speece, 1996). At pH value lower than 6.3 or higher than 7.8 the rate of methanogenesis decreases. From this reason, sodium bicarbonate ($NaHCO_3$) at a ratio of 0.5g$NaHCO_3$: 1 gCOD (Omil et al., 1999) was added in the influent to maintain the pH stability. At the end of the Experiment 4, pH was again dropped to the level lower than 6.3. The main reason for this pH drop would be that at higher amount of embalming fluid, more alkalinity was required. As not only COD was increased, at higher amount of embalming fluid the acidic phenol concentration was also increased. It was possible that alkalinity contained in the domestic wastewater was not sufficient at this embalming concentration. This assumption was supported by the lower alkalinity concentrations in the effluent from both reactors of Experiment 5 compared to those in the previous experiments (Figure 4.18). Moreover, decrease of influent temperature could attribute to this pH drop. The pH value in an anaerobic reactor was partly a function of temperature(Van Haandel A., 2007). This explains by the higher solubility of CO_2 at lower temperatures, which in itself would hydrolyses in the water and decrease the pH (Eq.4.1).

$$CO_2(aq) + H_2O \rightarrow H_2CO_3 \quad \text{(Eq.4.1)}$$

Another possibility was the pH might decreased if acid fermentation prevailed over methanogenesis. This was in accordance with the expected behavior of organic acids produced as intermediates in the process during unbalance could cause a rapid pH drop and cessation order of methane production. To correct the pH problem, higher amount of $NaHCO_3$ (1g $NaHCO_3$: 1g COD) was added. The pH was gradually bat steadily recovered to the average level of 6.8 at the end of Experiment 5.

4.2.3 Suspended Solid (SS) and Volatile Suspended Solid (VSS)

The average SS and VSS concentrations and removal efficiency during the steady state are presented in Table 4.2, while their concentrations throughout the experimental period are shown in Figure 4.20 to 4.23.

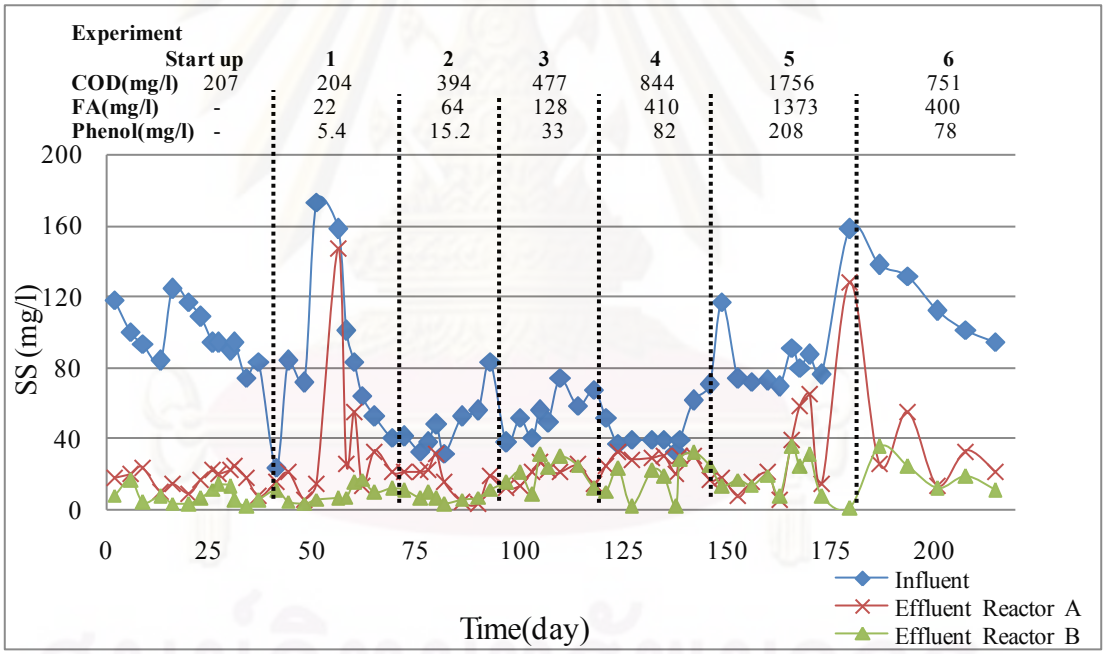


Figure 4.20 SS concentration during the whole experimental period.

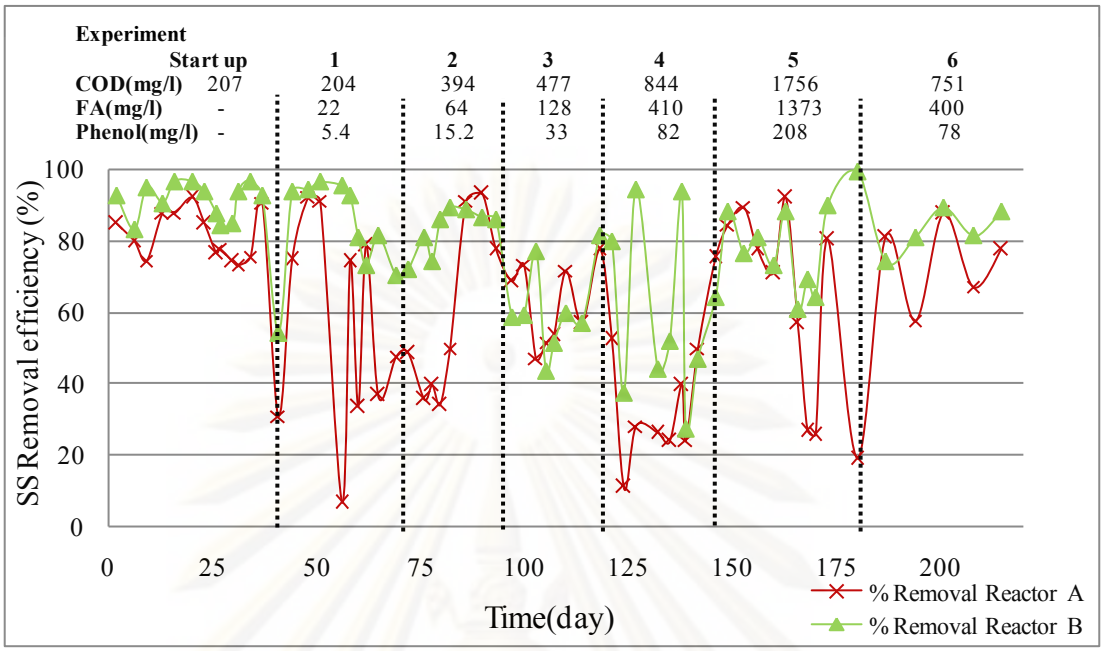


Figure 4.21 SS removal efficiency during the whole experimental period.

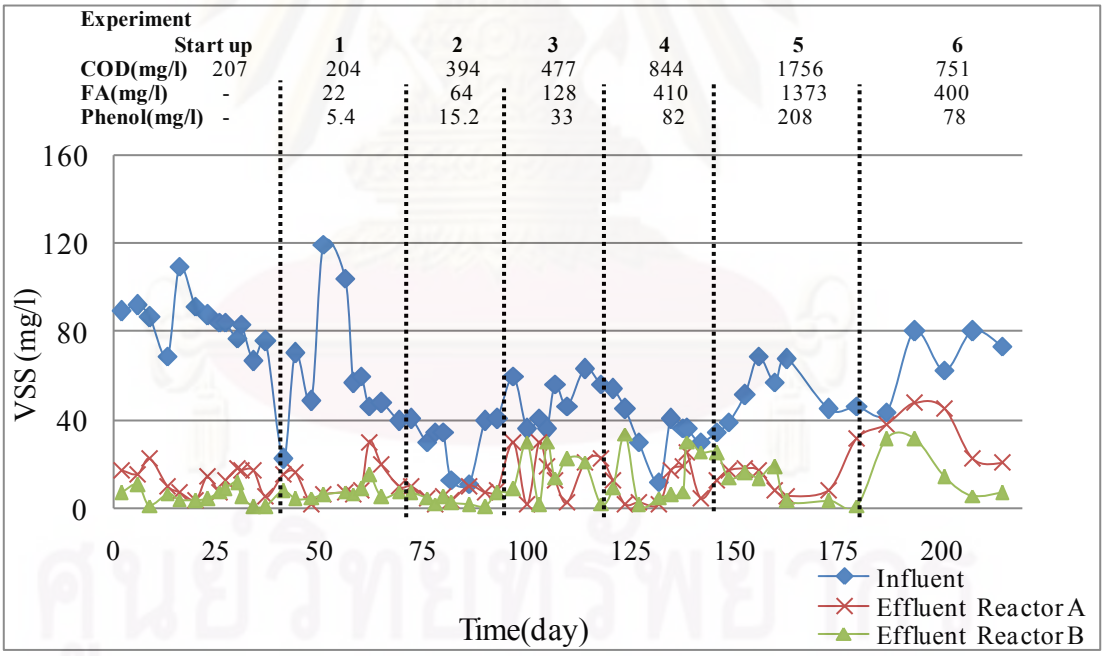


Figure 4.22 VSS concentration during the whole experimental period.

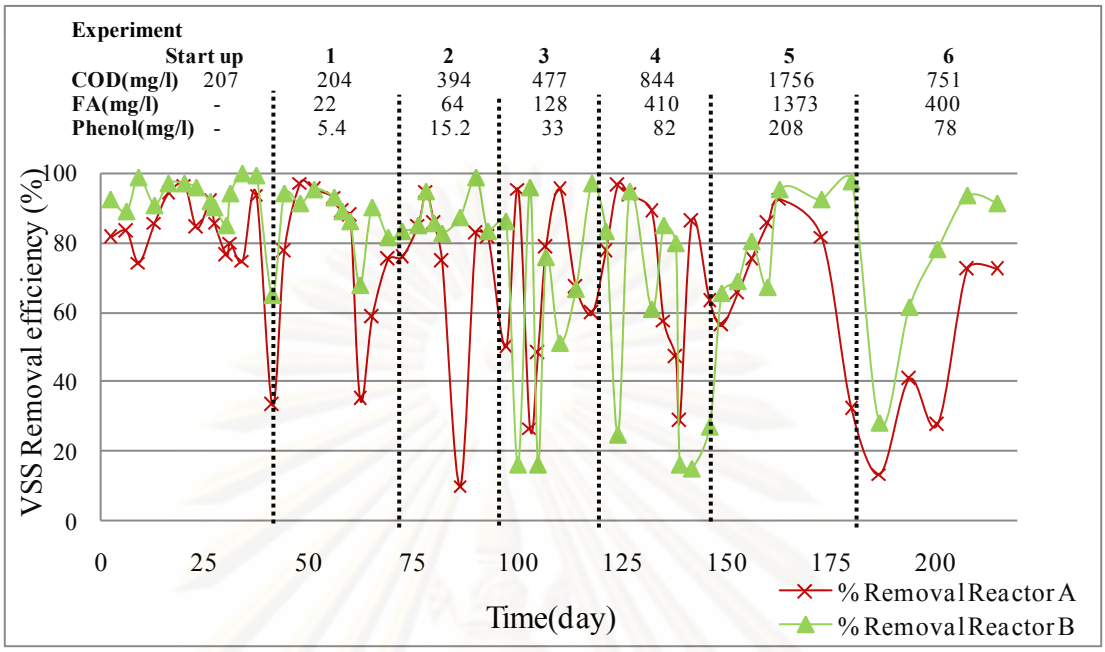


Figure 4.23 VSS removal efficiency during the whole experimental period.

Results in Figure 4.20 and Figure 4.22 show that concentrations of SS and VSS in the effluent were fluctuated greatly, depended on the quality of the influent sample. Without embalming fluid addition, both SS and VSS were removed rather consistently than when the reactors were fed with the mixture of domestic wastewater and embalming fluid. This implied that FA and phenol might affected the settle ability of sludge or deterioration of sludge floc was occurred upon exposed to the compounds. However, statistical analysis revealed the overall SS and VSS removal efficiencies of Reactor B were significantly higher than that of Reactor A ($P<0.05$) z Undoubtedly, this was the result of longer HRT of Reactor B providing longer time for solid to settle down.

4.2.4 COD

The average COD concentration and removal efficiency during the steady state are shown in Table 4.2, Figure 4.24 and 4.25 present COD concentrations and removal efficiency during the experimental period.

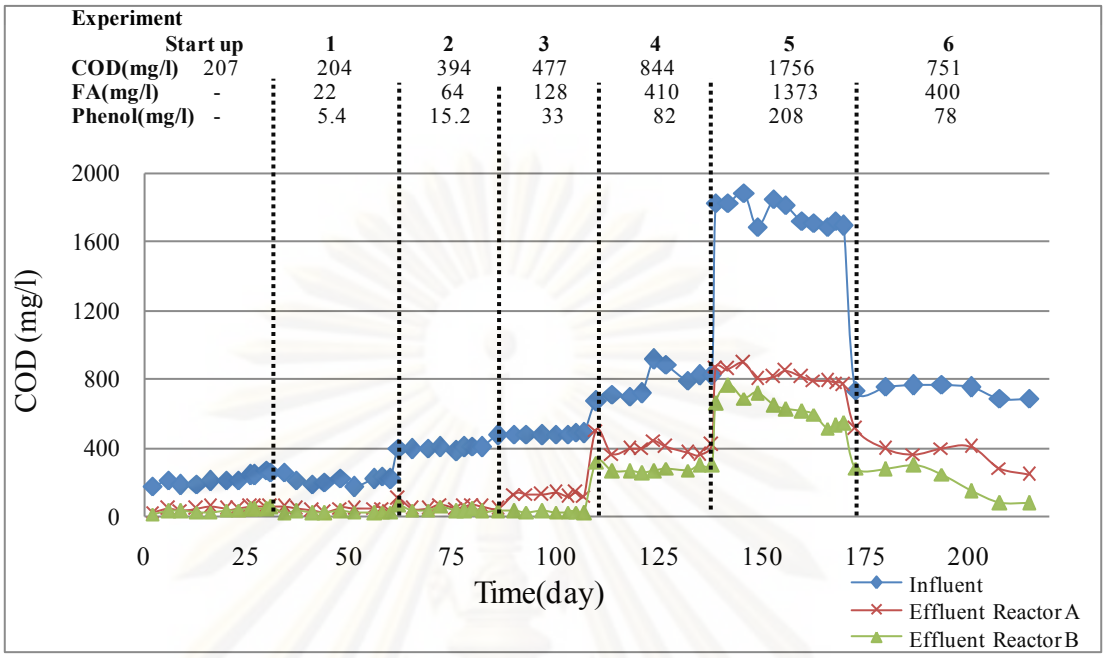


Figure 4.24 COD concentration during the whole experimental period.

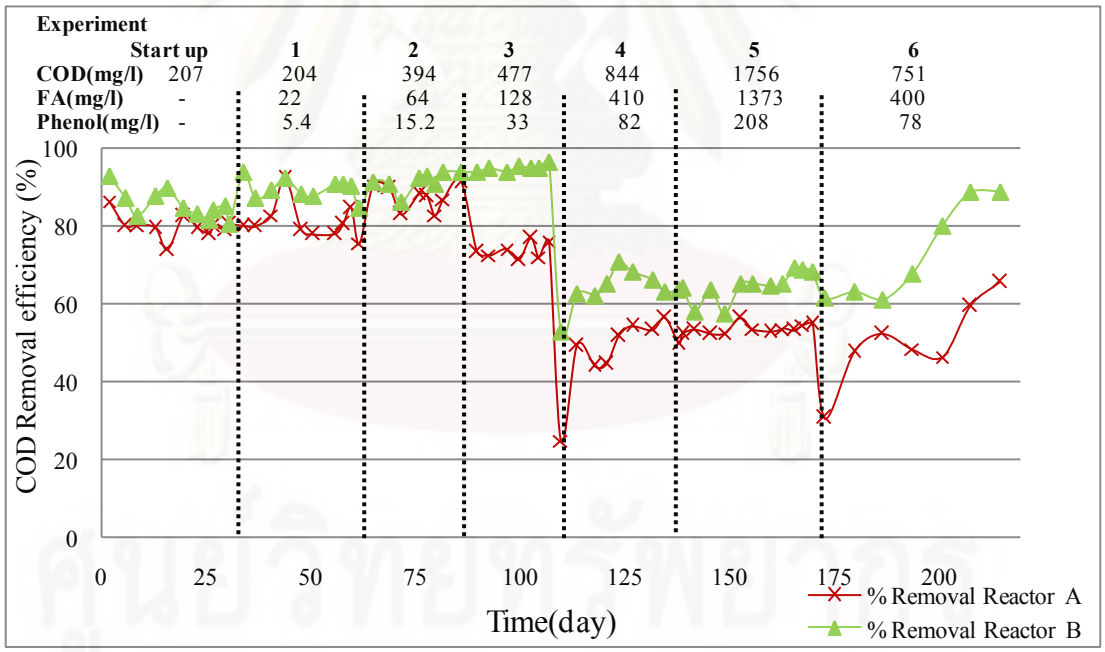


Figure 4.25 COD removal efficiency during the whole experimental period.

COD removal efficiencies were not affected after start feeding both reactors with the mixture of domestic wastewater and embalming fluid up to FA and phenol concentrations of 64 and 75 mg/l, respectively (Experiment 2). These confirmed by the fact that both reactors could efficiently remove COD after being fed with the embalming fluid immediately without the lag phase. The removal efficiencies

achieved from both reactors for Experiments 2 and 3 were not found to be significantly different ($P>0.05$). However, when COD of the influent was increased to 477 in Experiment 4, significantly higher COD removal efficiencies were observed in Reactor B compared to those in Reactor A ($P\leq0.05$). Moreover, the sudden drop of COD removal efficiencies were found at the Experiments 4 and 6 in Reactors A and B for a period 2-5 days before picking up to the steady state level. These results implied the inhibition effects and the acclimatisation of microbial activity when feeding with higher concentration of the toxic substances; FA and phenol. It was also found that reduced COD removal efficiencies were directly correlated to phenol concentration in the effluent. COD remained in the effluent was found to be fairly close to COD of phenol in the effluent, suggesting that both reactors could not efficiently removed phenol when present in the influent up to 33 and 82 mg/l for Reactors A and B, respectively.

4.2.5 FA

FA and phenol are the main compounds in the embalming fluid. FA, itself is a toxic substance but could anaerobically be removed (Omil et al., 1999; Vidal et al., 1999; Lotfy and Rashed, 2002; Moteleb et al., 2002; Oliveira et al., 2004; Pereira and Zaiat, 2009).

The average FA concentrations and removal efficiencies during the steady state are tabulated in Table 4.2. Graphs presenting FA concentration and removal efficiencies during the whole experimental period are shown in Figure 4.26 and 4.27.

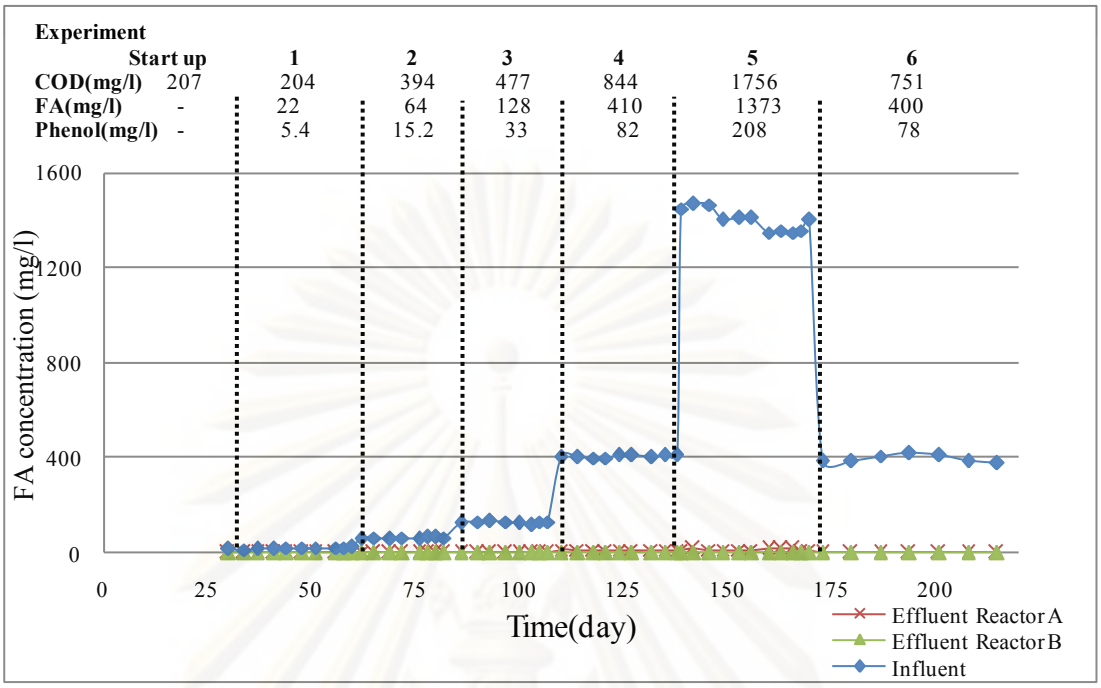


Figure 4.26 FA concentration during the whole experimental period.

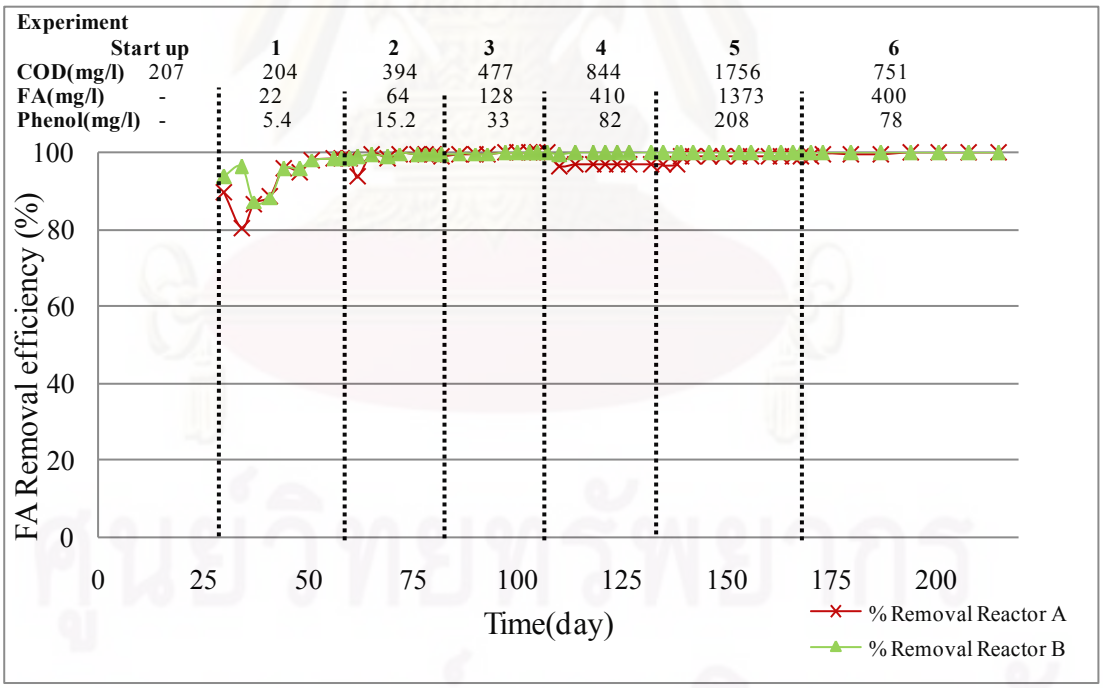


Figure 4.27 FA removal efficiency during the whole experimental period.

According to some previous studies, removal of FA by adsorption volatilisation in the bioreactor was considered negligible. This assumption was based on the study of Omil et al. (1999), who reported only 10-11% of abiotic formaldehyde removal in the anaerobic bioreactor. However, as the continuously shaken batch

experiment using fresh sterile sludge was utilised in Omil et al's study, percentage of abiotic FA removal especially via absorption, should be higher than that actually occurred in this current study. To ensure that volatilisation was not the removal pathway of FA in this current study, an experiment for determining the volatisation of the FA from the continuously stirred influence tank was performed. For this purpose, samples were collected from the influent tank in which embalming fluid was mixed with domestic wastewater throughout the period of 8 hours to determine FA concentrations. Results of this experiment are presented in Table 4.4.

Table 4.4 FA concentrations in samples taken from the influent tank during the 8 h period.

Time (hour)	FA concentration (mg/l)
0	1386
2	1298
3	1327
5	1314
6	1335
8	1353
Average	1336
SD	31.1
RSD(%)	2.3

Result from Table 4.4 indicated that FA concentrations measured in samples collected from the continuously stirred tank during the period of 8 h at the room temperature were not significantly different (RSD=2.3%). It could be, therefore confidently concluded that no valatilisation of FA measured from the influent tank before feeding into the anaerobic filter. Likewise, if volatilization of FA was not observed in the completely stirred influent tank, removal of FA in the laminar plug flow anaerobic filter would not surely be occurred. From all reasons mentioned above, it would be fair to state that any FA removal found in this current study was mediated by the biological activity.

Lag phases were observed after embalming fluid addition in Experiment 1 (Figure 4.27). After the lag phase, which took approximately 2 weeks, high FA

removal efficiencies (97.0 and 97.2 % in Reactors A and B, respectively) were achieved in both reactors. Very low FA concentrations were detected in the effluent samples during the steady state of Experiment 2, implying complete FA removal from the wastewater. Nearby complete FA removal was later found in all next experiments without the lag phase regardless of FA concentrations in the influent. This result indicated that anaerobic degradation of FA in anaerobic filter was very efficient for the studied range of initial FA concentrations (22.1-1755.8 mg/l). Additionally, the FA removal efficiencies obtained from both reactors were not statistically different ($P>0.05$).

Table 4.5 shows the maximum FA concentration applied to both studies reactors and the corresponding parameters.

Table 4.5 The maximum FA concentration and corresponding parameters

Reactor	Maximum FA Concentration (mg/l)	Organic Loading Rate (kg COD/m^3.d)	FA Loading Rate (kg FA/ m^3.d)
A (6h-HRT)	1373	2.56	2.74
B (12h-HRT)	1373	1.28	1.37

As FA was nearly completely removed, the maximum degradation capacity of FA in the system was still not exceeded. Similarly, Oliveira et al. (2004) reported that initial FA concentration of 1158.6 mg/l was completely degraded in the anaerobic filter operated at HRT of 4.8 hr (Table 4.6)

Table 4.6 Comparison of FA removal efficiency found in this current study and in some previous studies removal efficiency.

Type of reactor	Organic loading rate	FA (mg/l)	HRT	Efficiency (%)		Ref.
				COD	FA	
AF	1.28 kg COD/m^3.d 2.56 kgCOD/ m^3.d	1373	12 h 6 h	77	99	This current study
AF	5.78 kgFA/ m^3.d	26.2–1158.6	4.8 h	92	95	Oliveira et al. (2004)
UASB	6.0 kg COD/m^3.d	50-2000	14.4h	90-95	>95	Vidal et al. (1999)
UASB	0.37 -2.96 kg COD/ m^3.d	625–5000	1.8 d.		99.5	Eiroa et al. (2006)
Fluidized bed	5.1 kg COD/m^3.d	20-1100	8-16h		97.34	Moteleb et al. (2002)
Batch	3.75 kgCOD/m^3.d	0-300			49-90	Omil et al. (1999)
Batch		0-3000		92.4	28-98	Lu (1998)
ASBBR	0.08-2.78 kg FA/ m^3.d	36.1-1104.4	8 h	70.8	99	Pereira et al. (2009)
EGSB	10 kgCOD/m^3.d	200-1400				Gonzalez-Gil (1999)
Batch		31.5-125				Lotfy (2002)

As shown in the Table 4.6, FA removal efficiencies were very high especially when the continuously fed plug flow-reactors were utilized. On the other hand, the inhibition effect was observed in the batch-fed reactor operating at high concentration. Lotfy and Rashed (2002) utilised the batch reactor and observed that at FA concentrations higher than 200 mg/l methanogenesis was completely inhibited. A partial recovery of the bacterial activity was observed after 250 h when the FA had been removed from the medium. These results clearly indicated the reversibility of FA caused inhibition in batch reactor. In addition, Pereira & Zaiat (2009) supported

the idea that batch reactor was less suitable for complete formaldehyde degradation than the continuous immobilized-cell reactor. They suggested that in the batch reactor, the entire microbial community was subjected to primary substrates, byproducts and end products, increasing the possibility of activity inhibition. Whereas, in continuous-flow reactors especially those with flow pattern close to plug flow, specific biomass can grow along the reactor's length so that, in each segment, a group of microorganisms could be adapted to specific compounds optimizing the degradation of primary substrates and byproducts. However, many types of anaerobic reactor could be operated for formaldehyde degradation if the biomass was allowed to acclimatise (Lu and Hegemann, 1998; Vidal et al., 1999; Lotfy and Rashed, 2002; Moteleb et al., 2002; Oliveira et al., 2004; Pereira and Zaiat, 2009; Raja Priya et al., 2009).

Domestic wastewater used as co-substrate in this experiment possible also played an important role in the degradation of FA. As shown previously, co-substrate is an interesting option for improving yield of anaerobic degradation. Vidal et al. (1999) showed that higher FA concentrations were tolerated when they were added continuously to glucose enrichment systems rather than when slug doses were used. This also indicated that the continuous operation was more favourable for bacterial acclimation. In case of FA as a pure substrate, the bacterial activities were very slow at the beginning of the run and FA degradation was achieved rapidly by the adding of acetate as the co-substrate (Lotfy and Rashed, 2002).

4.2.6 Phenol

Phenol is toxic to several biochemical reactions. However, biological transformation of phenol to a non-toxic entity is possible though specialized microbes.

The average phenol concentrations and removal efficiencies during the steady state are shown in Table 4.2. Figure 4.27 and 4.28 show phenol concentrations and removal efficiencies throughout the experimental period.

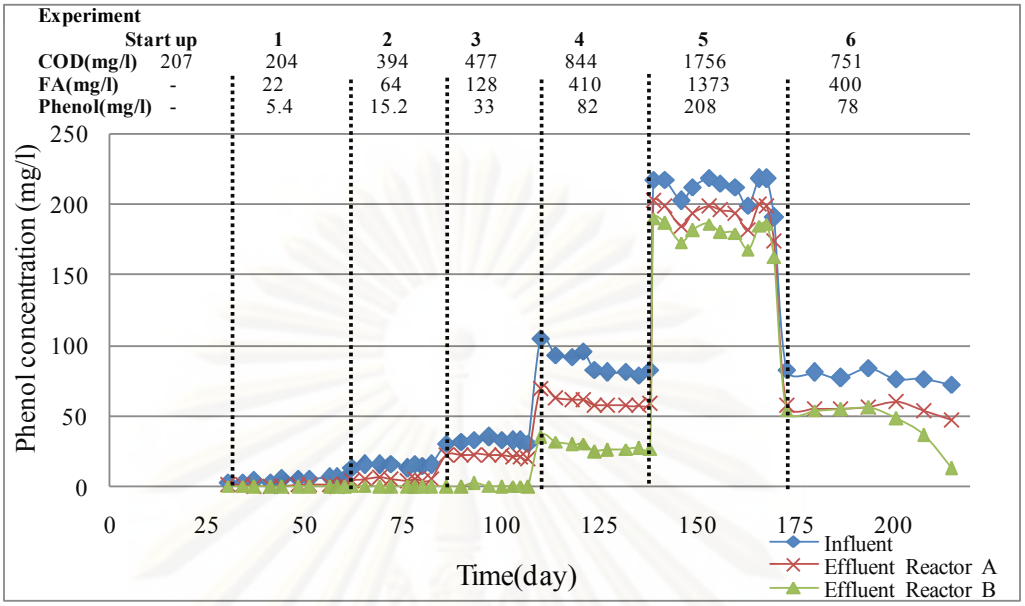


Figure 4.28 Phenol concentration during the whole experimental period.

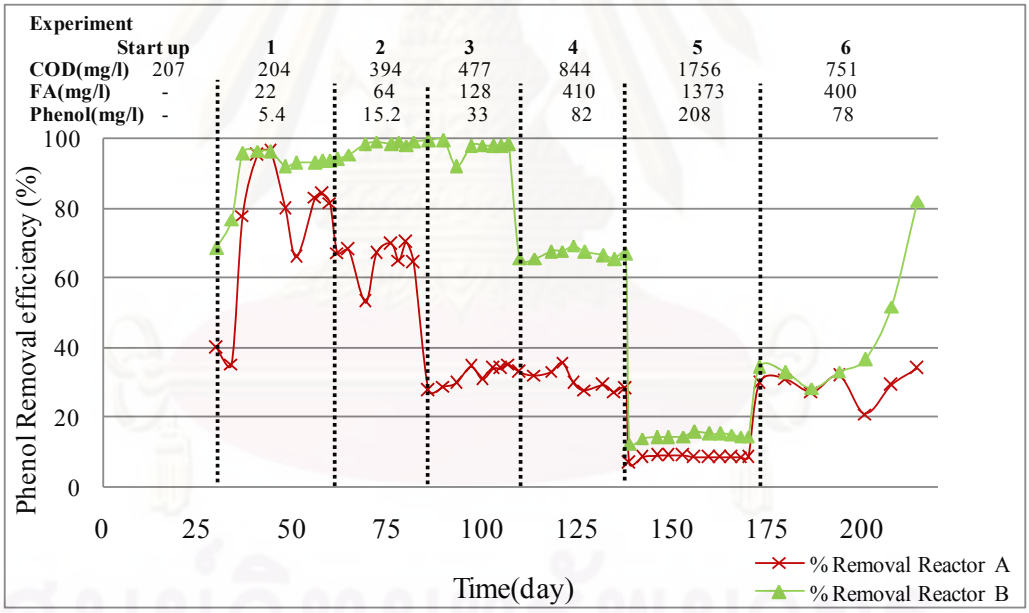


Figure 4.29 Phenol removal efficiency throughout the operation period.

The phenol removal efficiencies showed the same trend as that of COD. Increase of phenol concentration resulted in the decrease of both phenol and COD removal efficiencies. This indicated that majority of organic substances detected in the effluent were present in form of phenol. However, the significant higher phenol removal efficiencies was found in Reactor B than that of Reactor A ($P<0.05$). At the initial phenol concentration of 33 mg/l (Experimental 3), 98% was found to be removed in

Reactor B while only 32% was removed in Reactor A. This result showed the advantage of longer HRT in removing toxic substances (Speece, 1996). Phenol removal efficiencies above 95% were obtained from Reactor B at initial phenol concentrations of 5.4-33 mg/l. Afterwards, increase of phenol concentrationto 82 mg/l and 208 mg/l in the Experiments 4 and 5 resulted in phenol removal efficiencies being sharply reduced to 67.3 % and 15.1 % (Figure 4.25 and Figure 4.29). During this period, pH values of the effluent were slightly dropped (Figure 4.18). To solve this problem, $NaHCO_3$ was added in the feed to increase alkalinity level. Nevertheless, after $NaHCO_3$ addition, COD and phenol removal efficiencies were not improved. This result indicated that the biodegradation of phenol was completely inhibited. By decreasing the concentration back to 78 mg/l, the phenol removal efficiency could recover, judging from phenol removal efficiencies, after the period of 35 days with the tendency to increase to the level higher than that obtained from previously similar phenol concentration (Experiment 4), especially in Reactor B. According to Fang et al. (2006) the phenol treatment efficiency was recovered from the bioactivity inhibition to nearly 100% by reducing phenol loading rate. Based on high removal efficiency (more than 70%) maximum phenol concentrations that could be treated in the studied anaerobic filters and the corresponding parameter are shown in the Table 4.7.

Table 4.7 The maximum phenol concentration and corresponding parameters

Reactor	Maximum Concentration (mg/l)	Organic Loading Rate (kg COD/m^3.d)	Phenol Loading Rate (kg phenol/ m^3.d)
A	15.2	0.026	0.061
B	33	0.028	0.066

According to Eioa et al. (2005), removal of phenol by adsorption volatilisation in the bioreactor was considered negligible.

Table 4.8 Comparison of phenol removal efficiency found in this current study and in some previous studies removal efficiency.

Type of reactor	Organic loading rate	Phenol (mg/l)	HRT	Efficiency (%)		Ref.
				COD	Phenol	
AF	0.28 kgCOD/m^3.d 0.066 kgPhenol/m^3.d	33	12h	95	98	This current study
AF	2.03kgCOD/m^3.d	50-1200	12 h	98	99	Bolanos et al. (2001)
UASB	0.9kgCOD/m^3.d	630	40h	96	99	Fang et al. (2006)
EGSB	1.2kgPhenol/m^3.d	500		90	99	Scully et al. (2006)
UASB	6 kgCOD/m^3.d	1260	12 h		98	Ke et al. (2008)
Batch*	0.89 kgCOD /m^3.d	30-580			33.7-96	Eioa et al. (2005)
Batch**		11.8-1140				Lofty (2002)

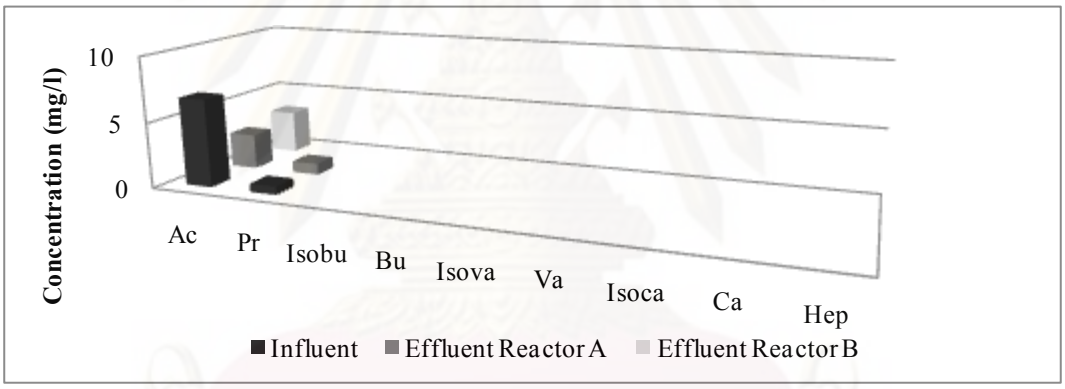
*FA concentration of 260 mg/l was used. , **The municipal wastewater contained either FA or phenol or the mixture of them.

The threshold OLR of 0.066 kg phenol/ m^3.d for effective phenol removal obtained in Reactor B (12h-HRT) was substantially lower than those reports in the literatures. This lower phenol loading rate could be explained by the presence of FA in the influent. Eiroa, et al. (2005) found that at the fixed initial concentration of 260 mgFA/l, the maximum phenol concentration that could be efficiently degraded was 180 mg/l. Additionally, Lofty and Rashed (2002) also reported inhibition of phenol degradation in the presence of FA. In this current study, 99% of phenol was removed in the Reactor B when 64 mgFA/l and 15.2 mgPhenol/l were containing in the influent. Moreover, when initial COD loading rates were increased to 1.6-1.7 kgCOD/m^3.d (Table 3.2), the phenol removal efficiencies were dropped to 65% in the Experiment 2 (Reactor A) and 4 (Reactor B). This indicated that, apart from the presence of FA, the COD loading rate also limited to the phenol removal efficiency of anaerobic filter.

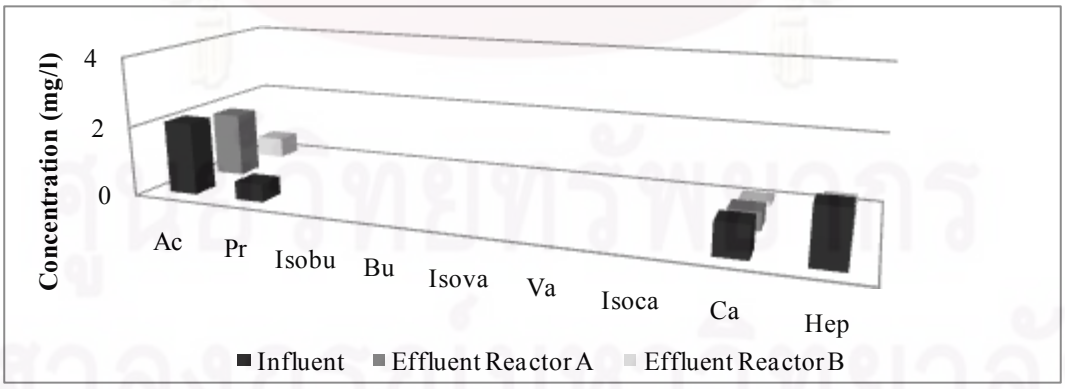
4.2.7 VFA composition

Acid-forming bacteria convert soluble organic compounds into VFA which, in turn, is used by the methane-forming bacteria. The VFAs measured in this study were acetic (Ac), propionic (Pr), iso-butysic (isobu), butyric(bu), iso-valeric (iso-va), valeric, iso-caproic (iso-cap), caproic (cap) and haptanioic (hep) acids.

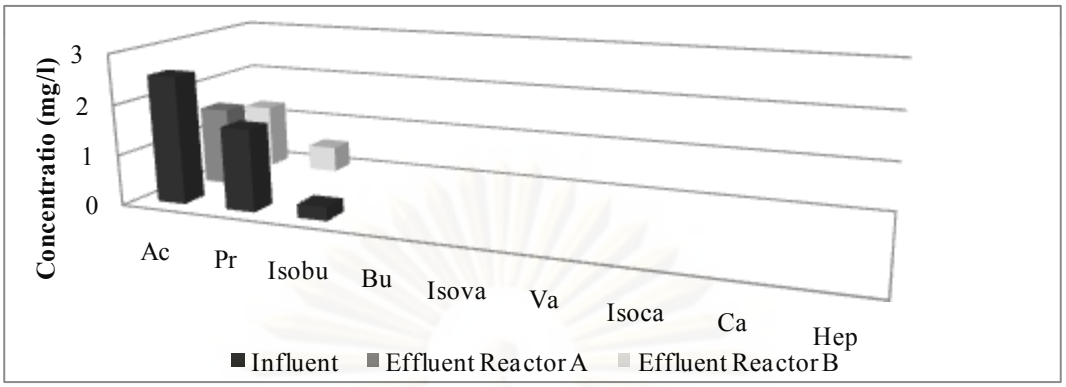
Less than 7mg/l of acetic acid was detected in influent and effluent samples when both reactors were fed with embalming fluid. In addition, low concentrations of propionic (max. 1.33 mg/L), isobutyric (max. 3.14 mg/L), butyric (max. 0.79 mg/L), isovaleric (max. 0.43 mg/L) valeric(max. 4.52 mg/L), iso-caproic (max. 0.63 mg/L), caproic (max. 0.56 mg/L) and haptanioic (max. 1.17 mg/L) acids were also detected. Very low concentrations of VFAs found in the effluent indicated that VFA were not accumulated in the reactor.



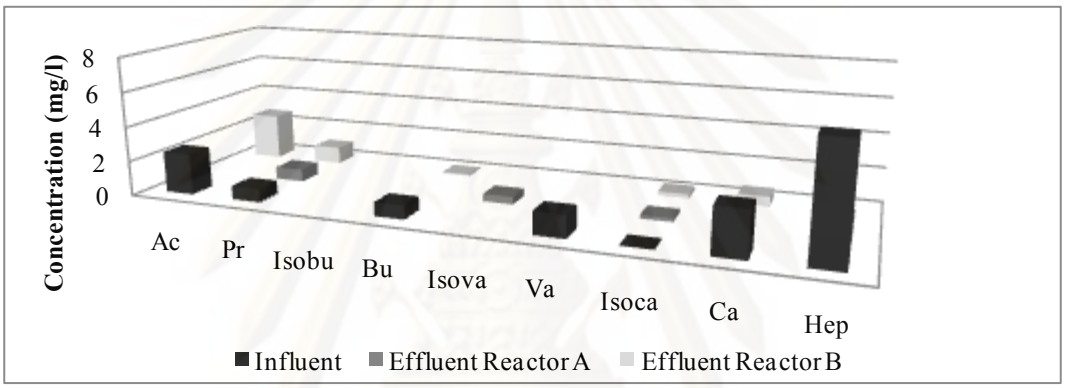
(a)



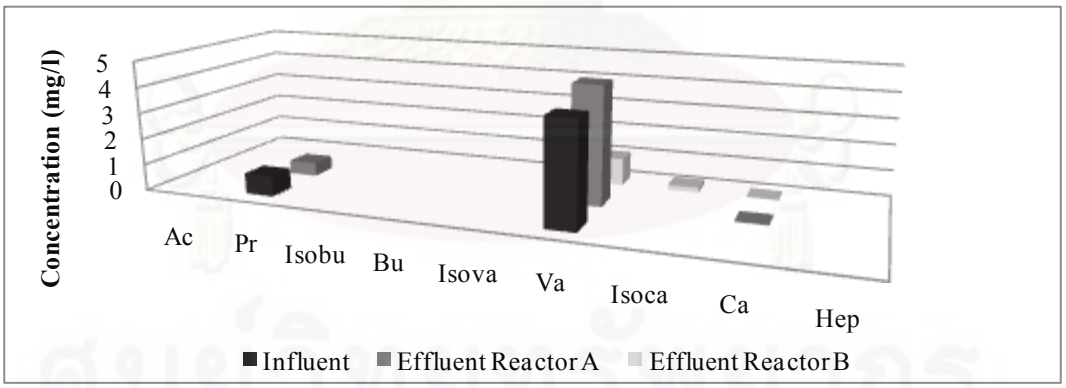
(b)


Concentratio (mg/l)
3
2
1
0
Ac
Pr
Isobu
Bu
Isova
Va
Isoca
Ca
Hep
Influent
Effluent Reactor A
Effluent Reactor B

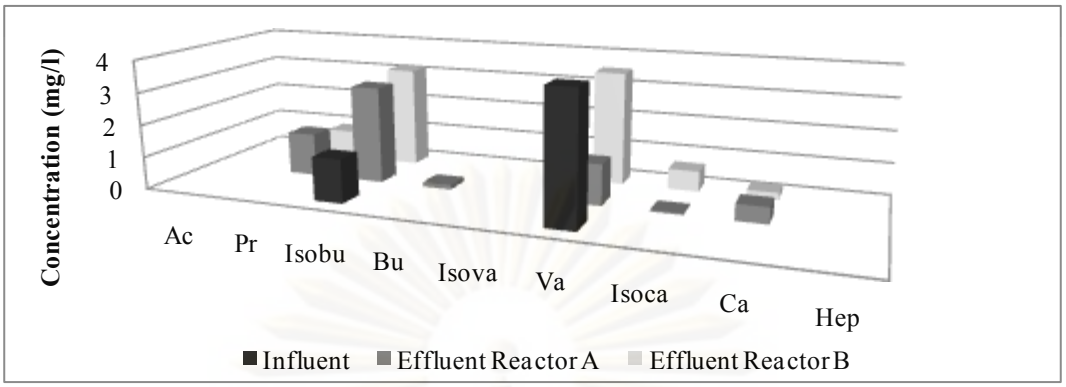
(c)


Concentration (mg/l)
8
6
4
2
0
Ac
Pr
Isobu
Bu
Isova
Va
Isoca
Ca
Hep
Influent
Effluent Reactor A
Effluent Reactor B

(d)


Concentration (mg/l)
5
4
3
2
1
0
Ac
Pr
Isobu
Bu
Isova
Va
Isoca
Ca
Hep
Influent
Effluent Reactor A
Effluent Reactor B

(e)



(f)

Figure 4.30 VFA composition: (a) :Experiment 1, (b) :Experiment 2, (c) :Experiment 3, (d) :Experiment 4, (e) :Experiment5, (f) :Experiment 6.

4.2.8 Gas volume and gas yield

The average gas volume and gas composition during the steady state were shown in Table 4.2. The gas volume and CH_4 yield gained throughout the experimental period are presented in Figure 4.31 and 4.32.

After embalming fluid addition, the gas production rate was immediately reduced, but FA and phenol conversion proceeded without the lag-period as has been shown previously in Topic 4.2.5 and 4.2.6. At the FA and phenol concentration of 22 and 5.4 mg/l (Experiment 1), the stability of biogas production was achieved after a lag phase of 7 or 8 days. This pattern was also found when increased concentration of FA and phenol were applied.

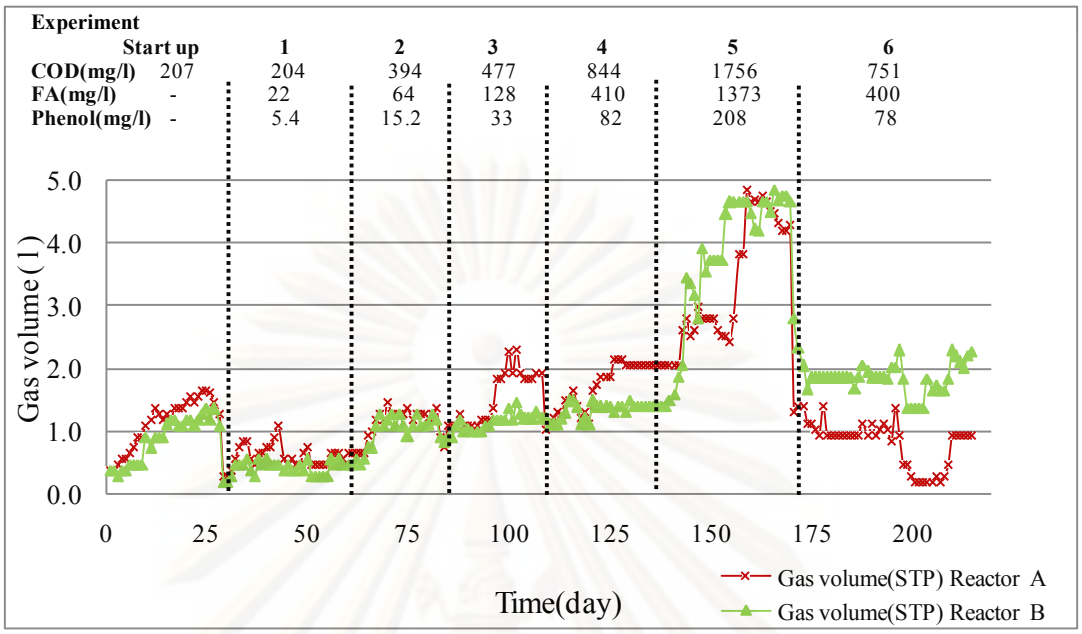


Figure 4.31 The volume of gas production during the whole experimental period.

Table 4.9 Methane compositions in the biogas during the whole experimental period.

Experiment no.	Reactor A	Reactor B
Start up	48±1.29	53±1.46
1	54±3.84	56±3.71
2	56±2.78	56±3.15
3	58±4.63	62±1.89
4	68±0.87	70±2.34
5	77±0.77	77±1.56
6	67±2.55	73±4.04

Presence of embalming fluid caused the decrease of the specific methane yield (Figure 4.32). These phenomena could possibly be attributing to the decrease of microorganism activity from the acute toxic of FA and phenol. Lu et al. (1998) observed the decrease of anaerobic gas production when concentration of FA was increased. In addition, according to Fang et al.(2006), bioactivity was partially inhibited by the increased phenol-loading rate. The results of the gas production and specific methane yield inhibition observed in this study was not mentioned in

previous studies conducted by Oliveira et al. (2004) and Moteleb et al. (2002), when the anaerobic filter was used for treating either FA or phenol as sole substrate. Hernandez and Edyvean (2008) found that at phenol initial concentration of 120.63 mg/l, phenol can inhibit both gas production and biodegradability. Gonzalez-Gil et al. (1999) report that at initial dose of 1400 mg COD/l, FA was highly toxic and no methane production was observed. The irreversibility reflected by the loss in the methane production rate could be the result of deterioration of to biomass.

Higher values of the specific methane yield observed during steady state of Experiment 5 compared to those gained from other experiments after embalming fluid addition, might partly be explained by the high CH_4 composition during this period. Relatively lower temperature could promote the hydrolysis of CO_2 in the gas phase into the liquid phase. Relatively higher CH_4 percentage found in Experiment 5 was possibly caused by new GC column being installed.

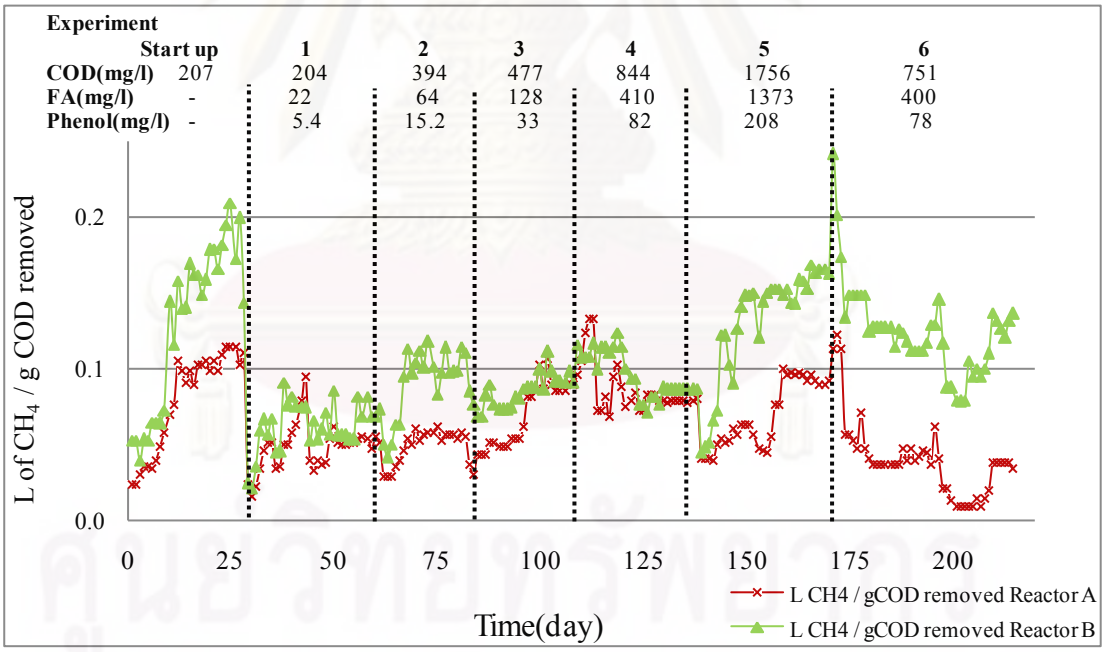


Figure 4.32 Specific methane yield (L CH_4/gCOD removed) throughout the operation period.

4.2.9 The maximum treatable ratio of embalming fluid to domestic wastewater

Based on the experimental results when high (>70%) removal efficiencies of phenol were achieved, the maximum ratio of embalming fluid to domestic wastewater which could be efficiently treated in the anaerobic filters were 0.002 : 1 and 0.004 : 1 by volume at the HRTs of 6h and 12h, respectively. Both of these ratios and HRT are used in the treatment system requirement estimation presented in Topic 4.4. The treatable FA and phenol concentration are shown in the
Table **4.10**.

Table 4.10 FA and phenol concentrations of each maximum treatable ratio

Maximum treatable ratio	Operation HRT (hr)	FA concentration	Phenol concentration
0.002 : 1	6	64	15
0.004 : 1	12	128	33

4.3 Microbiological observations and analysis

One of the key factors in the success of microbial-mediated process is an adequate understanding of process microbiology, more specifically the study of microscopic organism involved in waste degradation and by product formation. Owing to the constraint of sludge sample taking and Tay (1999)'s finding, in which the attached microorganisms were found to be relatively less important, only the suspended sludge was collected and analysed in this current study.

4.3.1 The physical characterisation of biomass

The biomass samples were observed via microscope for microbiological characterisation at the end of each operation with difference FA and phenol concentrations. In all operating conditions; rods, cocci and filaments with indentations at septa predominated (Table 4.9 and Figure 4.33). In addition, methanogenic archaea were observed along the reactor height and throughout the experimental period.

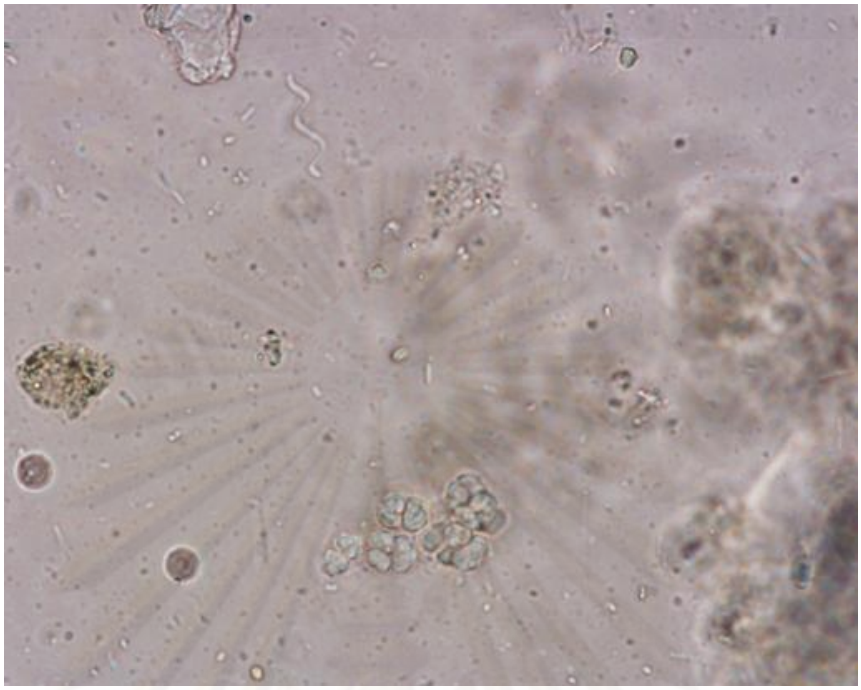
Table 4.11 (a) The microscopic observation of sludge from Reactor A

Experiment	Start-up	1	2	3	4	5	6
Bacteria							
-Rod	+ +	+ +	+ +	+ + +	+ + +	+ + +	+ + +
-Cocci	+	+	+	+	+	+	+
-Filament	+	+	+	+	+	+	+
-Helices	+	+ +	+ +	+ +	+ +	+ +	+ +
Achaea							
-*Methanosaeta* sp.	+	+ +	+ ++	+ +	+ +	+ +	+ +
-*Methanosarcina* sp.	-	+ +	+ + +	+ + +	+ + +	+ + +	+ + +

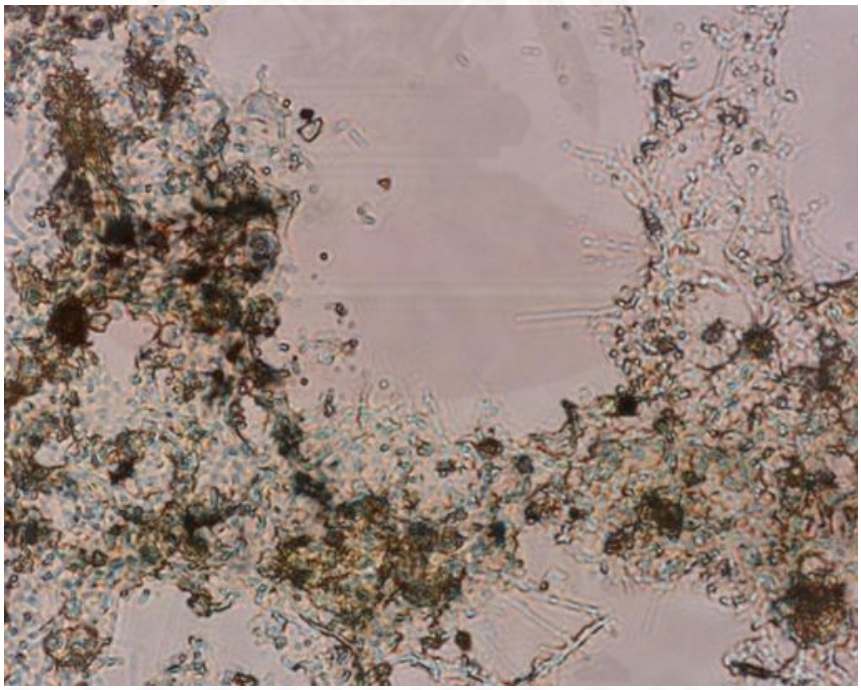
Table 4.11 (b) The microscopic observation of sludge from Reactor B

Experiment	Star-up	1	2	3	4	5	6
Bacteria							
-Rod	+ +	+ +	+ +	+ + +	+ + +	+ + +	+ + +
-Cocci	+	+	+	+	+	+	+
-Filament	+	+	+	+	+	+	+
-Helices	+	+ +	+ + +	+ + +	+ + +	+ + +	+ +
Achaea							
-*Methanosaeta* sp.	+	+ +	+ +	+ + +	+ +	+ +	+ +
-*Methanosarcina* sp.	-	+ +	+ + +	+ + +	+ + +	+ + +	+ + +

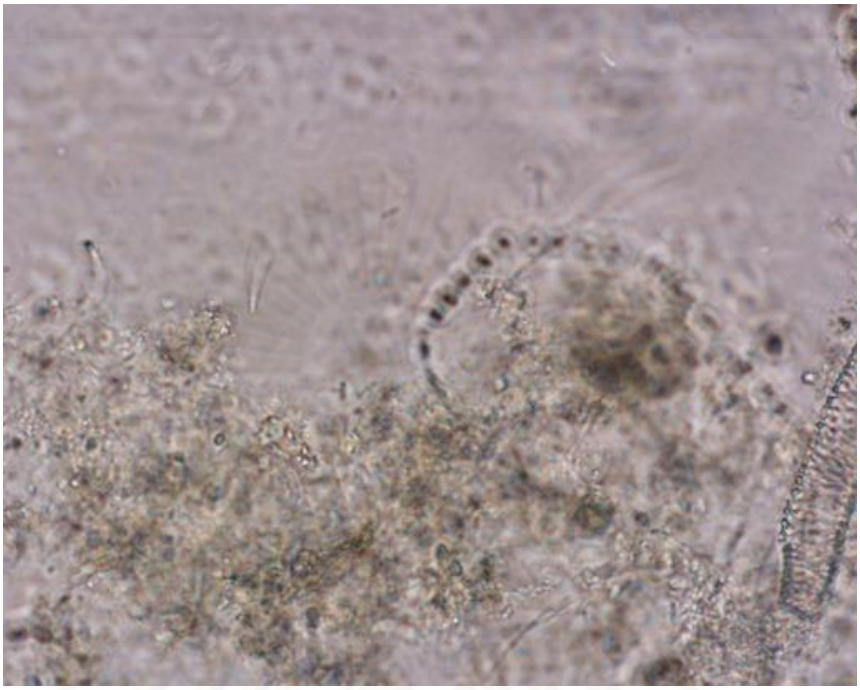
Note: + + +: Predominant, + +: frequent, +: rare, - : absent.



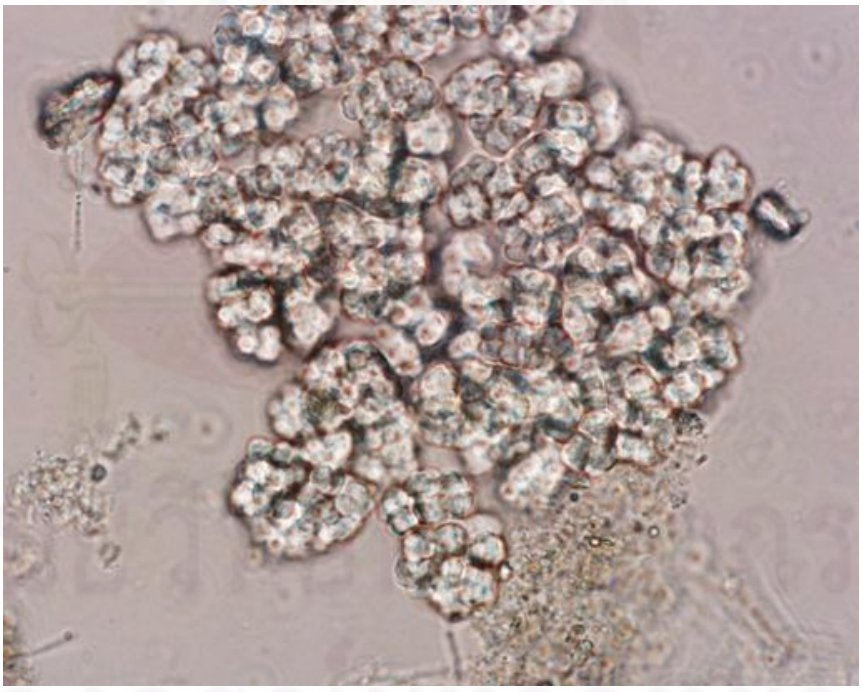
(a)



(b)



(c)



(d)

Figure 4.33 Phase-contrast photomicrograph of bacteria and methanogenic archaea in biomass samples. (a) Helices and rod bacteria (b) Filament bacteria (c) *Methanosaeta*-liked (d) *Methanosarcina*-liked

Rods, cocci, filament and helices were randomly distributed in the studied anaerobic filter. Among the methanogenic archaea, the organisms similar to *Methanosarcina* and *Methanoseata* dominated for all studied influent FA and phenol concentrations.

Like in anaerobic filter fed with FA or phenol (Bolanos et al., 2001; Oliveira et al., 2004), the configuration of the reactor, type of support material and concentration of acetate probably favoured the growth of such archaea cells. In the anaerobic filter treating FA (Oliveira et al., 2004), both *Methanosaeta* and *Methanosarcina* were found to prevail when influent FA concentrations were higher than 394 mgHCHO/L. Gonzalez-Gil et al., (1999); however, verified the prevalence of *Methanosarcina* in EGSB reactor treating FA at the range of 200-1400 mg/l. The sludge used by this research group was adapted to methanol and it was found that a portion of the FA was quickly transformed to methanol. Gonzalez-Gil et al., (1999), concluded that presence of methanol probably favoured the growth of Methanosarcina. On the other hand, Bolanos et al. (2001) reported on the presence of rods , cocci and *Methamosaeta*-like archaea in anaerobic filter treating phenol at the maximum concentration of 1,200 mg/l. However, in the batch reactor reported by Pereira & Zaiat (2009), *Methanosaeta*-like cells were rarely observed, which was in disagreement with the observations in the anaerobic filter. The good performance of the anaerobic filter is attributed to the presence of this microorganism, which is capable of consuming organic acids (Oliveira et al., 2004). This difference in microorganism populations can be related to the different configuration of reactors, and the fact that *Methanosaeta* sp. is more sensitive to FA and phenol inhibition (Bolanos et al., 2001; Oliveira et al., 2004). The anaerobic filter reactor, therefore, seems to be more favorable for the development of segmented biomass along the reactor's height (Pereira and Zaiat, 2009).

Organisms similar to *Methanosaeta* probably prevailed in the preferential use of acetate, while *Methanosarcina* preferentially used the methanol. Most species can also use H_2 or CO_2 and acetate. However, if cells are grown in the presence of both H_2 or CO_2 and acetate, the H_2 or CO_2 is used first, followed shortly thereafter by the acetate. Acetate is degraded by the aceticlastic reaction, with the methyl group reduced to CH_4 and the carboxyl group oxidized to CO_2 (Madigan et al., 2008).

However, *Methanosaeta* and *Methanosarcina* are the only organisms capable of using acetate to produce CH_4 and CO_2. The other archaea can only oxidize H_2 with CO_2 as the electron acceptor to produce CH_4 (Metcalf&Eddy, 2004). Other anaerobic microorganisms (helices, rod and filament bacteria) participated in the degradation of the FA and phenol of their polymeric products and of the long chain organic acids (Khanal, 2008). This current study confirmed the potential for anaerobic degradation of FA and phenol and also confirmed that microbial acclimation towards was important for the efficient toxic compounds removal.

4.3.2 DNA extraction

As mentioned in the Topic 3.7.3, the DNA could not be extracted from samples preserved using the absolute ethanol at the ratio of the sample: ethanol equal to 1:1 by volume (Figure 4.34 a.). Using the same samples fixed without ethanol addition (Figure 4.34 (b)), could be observed clearly on the agarose gel. The high DNA yield and high DNA purity were attained suggesting that the DNA extraction procedure utilised was suitable and efficient.

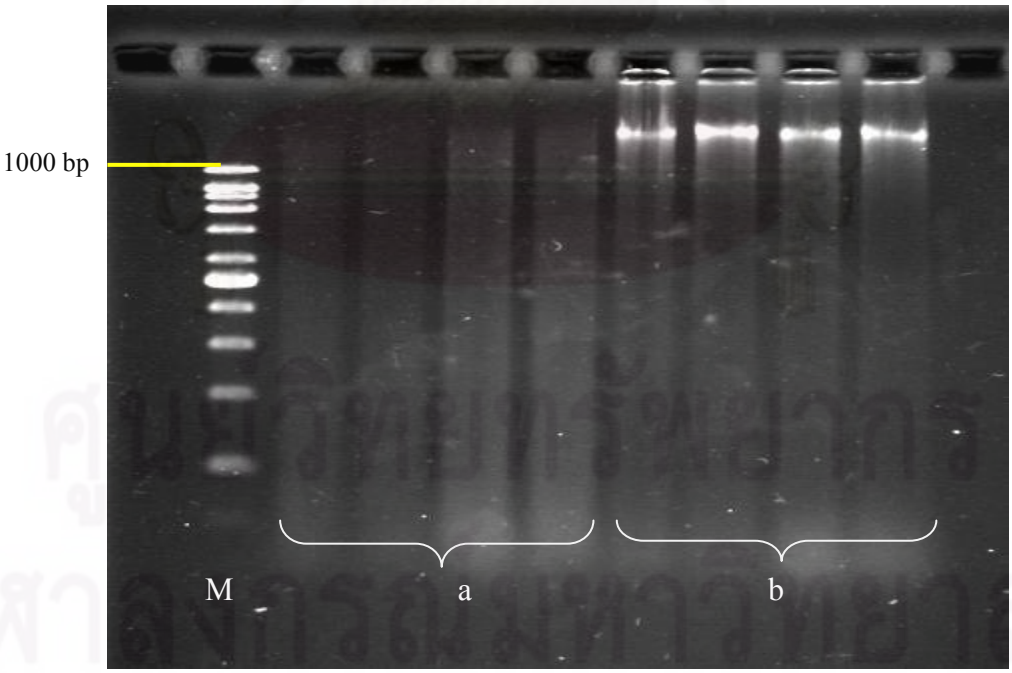


Figure 4.34 DNA extracted from the samples; M: marker 1kb, a: DNA extracted from ethanol-fixed samples, b: DNA extracted from the frozen non ethanol-fixed samples

4.3.3 PCR

PCR amplification of the 16S rRNA from the DNA extracted using ratio of 1/100 dilutions template proved to be reliable and produced high yields of PCR products for both archaea and bacteria (Figure 4.35-4.37).

PCR amplification was used to amplify the archaea population. After the first round of PCR amplification using the 46F/1100R primers, 1079 base pairs products were observed on agarose gels (Figure 4.36), indicating that the archaea comprised a large fraction of the biomass community. Then, these PCR products were amplified further using primers 340FGC/519R for specific archaea community. The 179 base pairs were produced (Figure 4.36) and used afterward for the DGGE analysis.

The 46F/1100R primers were used to amplify the PCR product of bacteria (Figure 4.37). These PCR products were chosen to be used for the DGGE analysis.

Figure 4.35 The 1079 base pair PCR product from the archaeaprimer 46F/1100R compared with 100 base pairs DNA marker (**Fermentas™**)

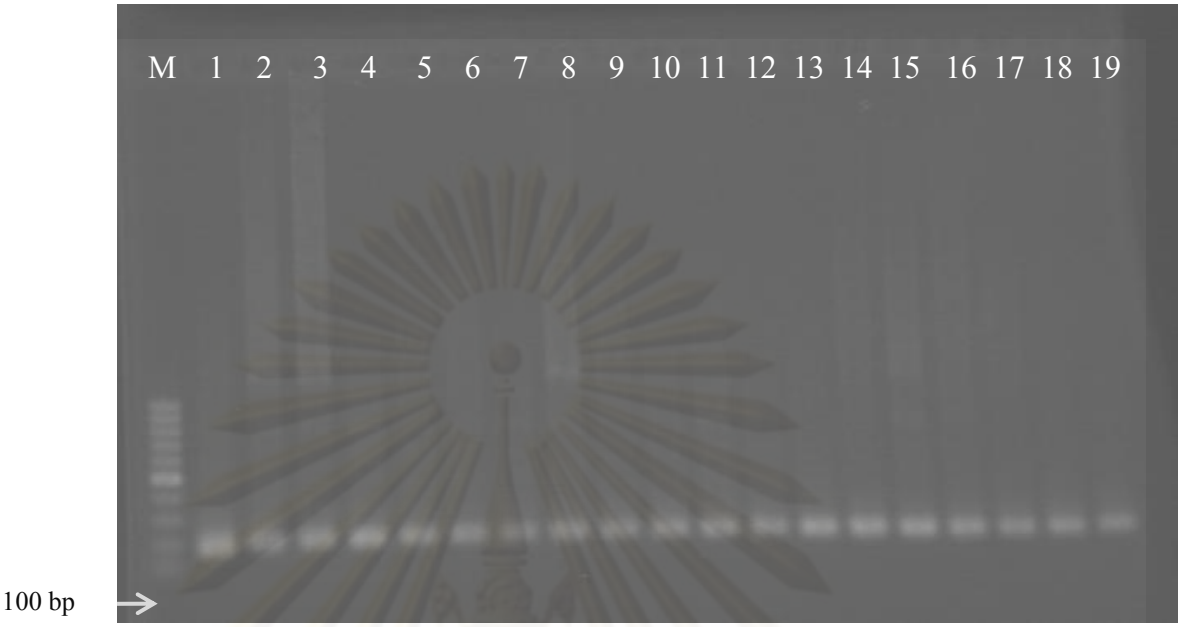


Figure 4.36 The 179 base pairs PCR product from the archaea primer F340GC/ R519 compared with those of the 100 base pairs DNA marker (**Fermentas™**)

Figure 4.37 The 149 base pairs PCR product from the bacteria primer F341GC/ R534 compared with 100 base pairs DNA marker (**Fermentas™**)

4.3.4 DGGE

The most important application of DGGE is in monitoring dynamic changes in microbial communities, especially when many samples need to be processed. The number of bands corresponds to the number of dominant species. This method can

give a good overview of the composition of a given microbial diversity (M.J. McPherson et al., 1996).

The DGGE profiles of archaea communities of samples collected from the studied anaerobic filters are shown in the Figure 4.38 and 4.39.

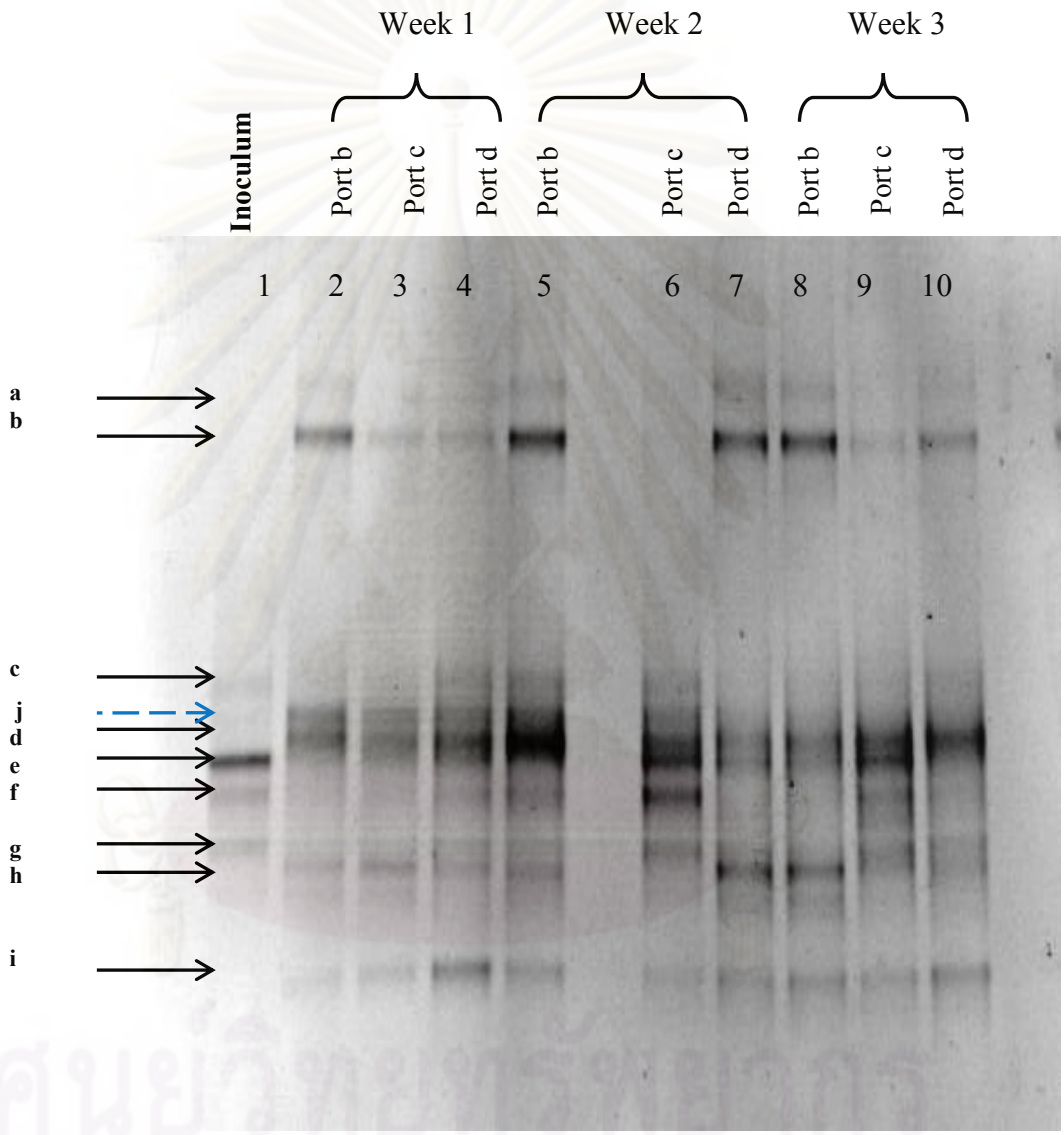


Figure 4.38 DGGE profile of archaea communities from Reactor A

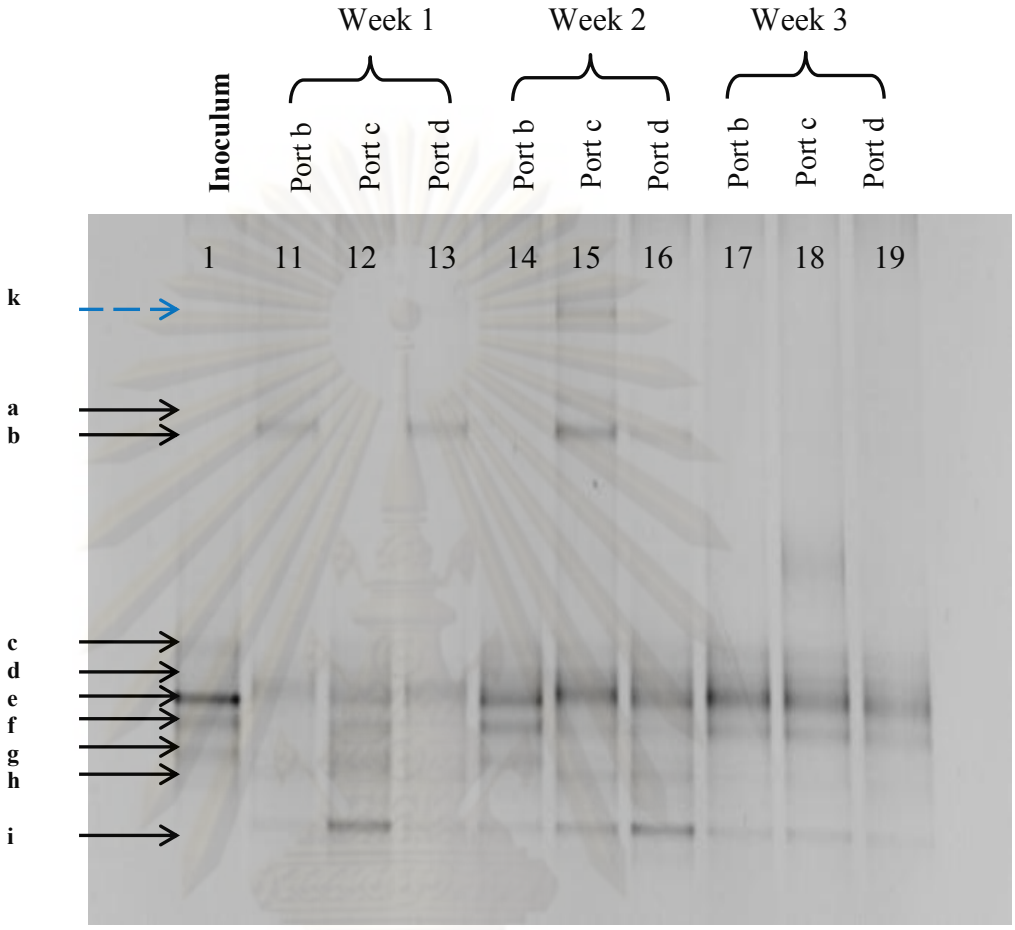


Figure 4.39 DGGE profile of archaea communities from Reactor B

DGGE profiles of archaea communities of the same reactor at different heights and time period showed difference band patterns. Appearance of some bands in samples from both reactors (Bands a-i), implying the common archaea community in the samples. After embalming fluid addition, Band c was lost from the inoculum communities, while the appearances of some new bands (Bands a, b, i, j, and k) were observed. Changing of DGGE profiles revealed alterations of archaea community in response to FA and phenol in the influent. Not only loss and appearance of band observed, changes of band intensity, which corresponded to changes of amount of particular species, were also visualized. Intensity fading of Band e during the first week after FA and phenol addition might be explained by the toxicity of these compounds to this specific archaea species. Moreover, there were bands which appeared only each reactor, e.g. Band j in the Reactor a or Band k in the Reactor B.

Different operating HRTs might play a part in the existence of these archaea species inside each reactor.

The DGGE profiles of bacteria communities (Figure 4.40 and 4.41) collected from different heights or time periods were also different. New bands (Bands c, e, g, i, j, k, and l) appeared after both reactors were fed with FA and phenol might be those new species adapting for both compounds degradation. The loss of Band f originally presented in the inoculum implying that some bacteria species might be damaged and eliminated. Moreover, the DGGE profiles obtained in samples from different operation HRTs seem to be dissimilar. This indicated that different HRT might affected communities of dominant bacteria species. Interestingly during week 3, when phenol removals were significantly improved (Figure 4.29), Bands c and k appeared on the gel of Reactor B with relatively high band intensity. At the same time, Band l which was not found in any other previous samples was also appeared at Port b of this reactor. This possibly showed adaptation and development of bacteria for treating FA and phenol resulting in higher removal efficiencies attained during this period than those previously detected.

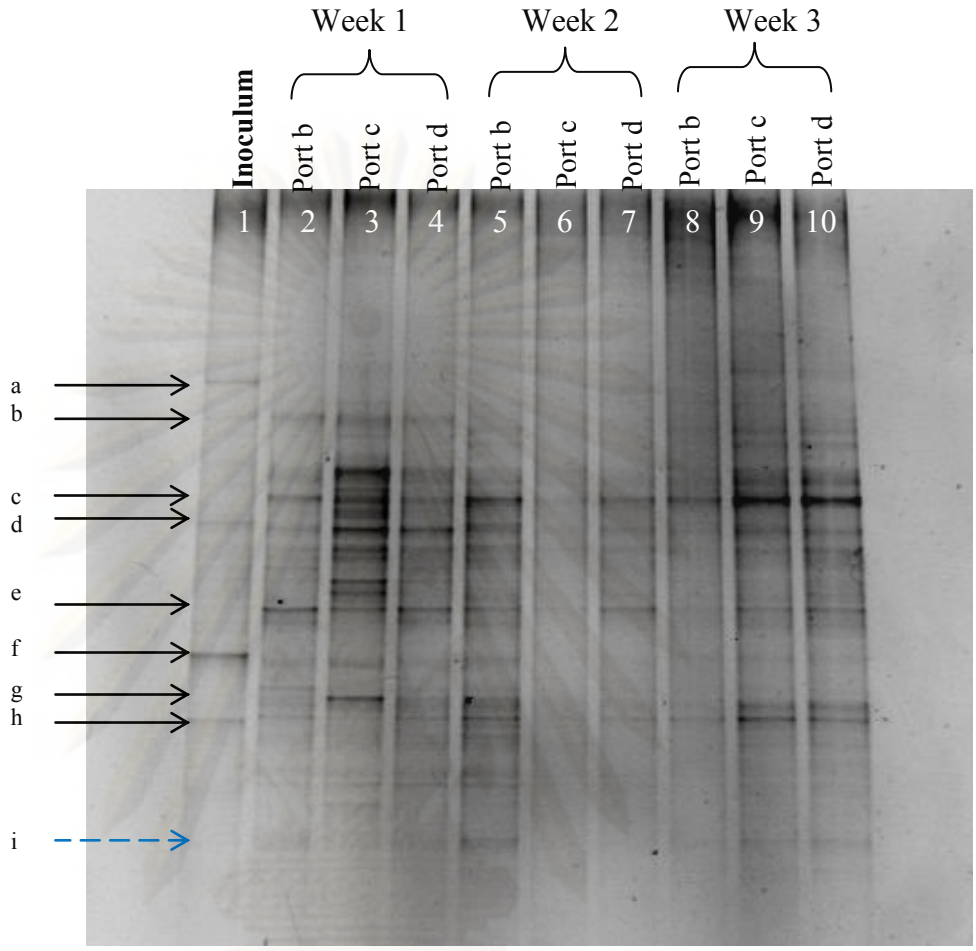


Figure 4.40 DGGE profile of bacteria communities from Reactor A

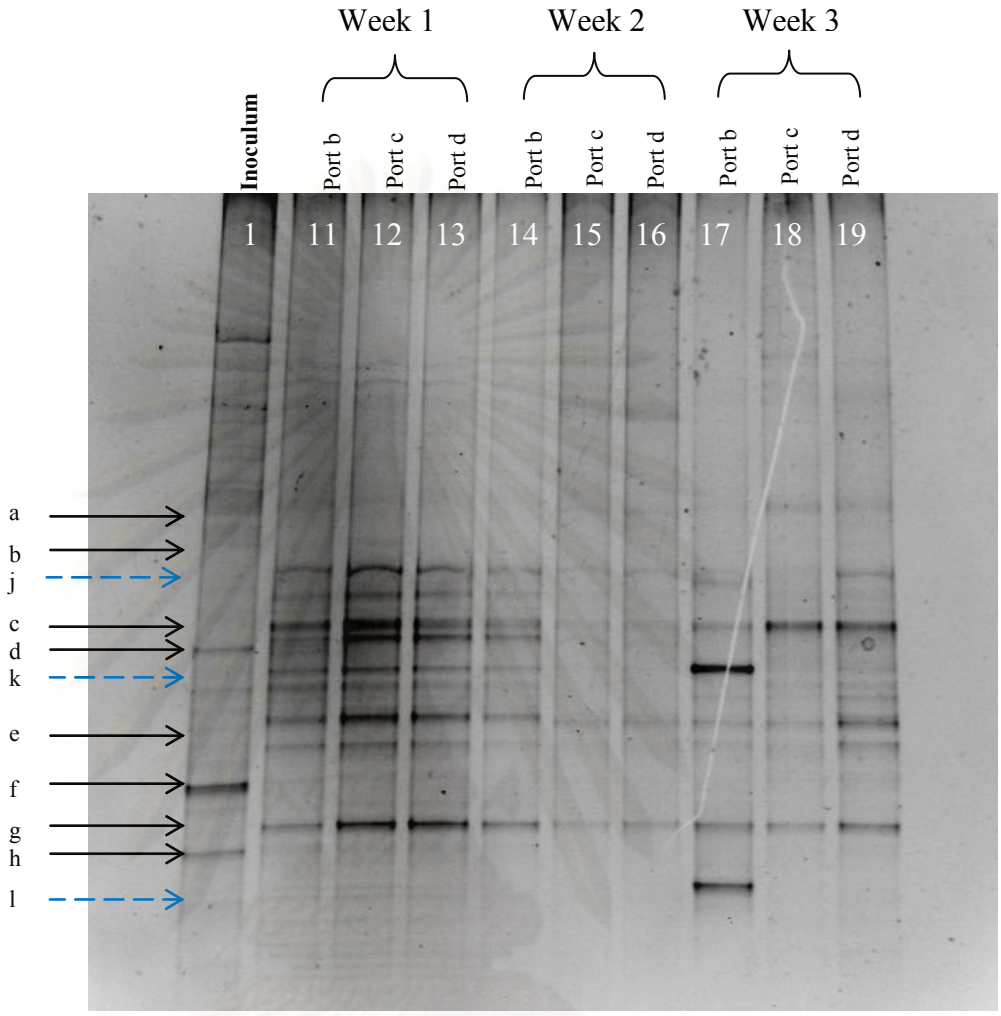


Figure 4.41 DGGE profile of bacteria communities from Reactor B

4.4 Case study: Treatment of embalming fluid by using domestic wastewater as co-substrate in full-scale anaerobic filter: Chaing Mai University's case.

Anatomy lab in Maharaj Nakorn ChiangMai hospital has generated the wasted embalming fluid around 15 m^3/year. To legally eliminate this liquid, the hospital spent approximately 20,000 Bath/m^3 of embalming fluid or around 300,000 Bath/year. The result from this study showed that anaerobic filter could be used to treat this embalming fluid using the domestic wastewater from the campus as the co-substrate.

Base on the lab-scale study, the full-scale anaerobic filter should be operated at 12h-HRT with the maximum embalming fluid to domestic wastewater ratio of 1 : 250 by volume, corresponding to the organic loading rate of 0.96 kgCOD/m^3.d. Using

the aforementioned design parameters, the required total volume of the full-scale anaerobic filter is 5.5 m^3. To attain the desired waste ratio, 44 l/d of wasted embalming fluid must be mixed with 11,000 l/d of domestic wastewater to form the influent for the reactor (at this ratio 128 mg/l FA and 33 mg/l phenol).

The constructed anaerobic filter would produce total biogas of 544 l/d, calculated from 0.1 lCH_4 / gCOD removed and 70% of CH_4 in the biogas. This amount of biogas is equivalent to 0.25 kg of Liquid Petroleum Gas (LPG) per day, which can be used as the supplementary source of energy especially in form of direct heat.

CHAPTER V

CONCLUSIONS

Based on the obtained results from the steady of effects of organic loading rate and hydraulic retention time on anaerobic filter performance in removing formaldehyde and phenol, the main ingredients of embalming fluid, the following conclusions could be drawn.

1. Anaerobic filter could completely remove FA at all studied initial FA concentrations (22-1373 mg/l) while phenol was completely removed when its concentrations were lower than 15 mg/l and 33 mg/l (equivalent to 0.061 and 0.066 kgPhenol/m^3.d) at the HRTs of 6h and 12h, respectively. It was possible that high concentrations of FA affected some microorganism responsible in degrading phenol. Moreover, the initial COD loading rate of 1.6-1.7 kgCOD/m^3.d also limited to the phenol removal efficiency of anaerobic filter.

2. Extended HRT positively affected anaerobic filter's performance in removing FA and phenol. Anaerobic filter operated at 12h-HRT could handle higher concentration to that at 6h-HRT. Moreover, significantly higher phenol removal efficiency was attained at longer HRT at the presence of higher FA concentration.

3. Presence of embalming fluid caused the decrease of the specific methane yield. It could possibly be attributed to the decrease of microorganism activity by the acute toxic of FA and phenol.

4. The maximum treatable ratio of embalming fluid to domestic wastewater were found to be 0.002 : 1 and 0.004 : 1 by volume at the HRTs of 6h and 12h at HRTs, respectively.
5. Microscopic pictures revealed the rods, cocci, filament and helices microorganisms as the dominant microbial species inside the studied anaerobic filters. Among the methanogenic archaea, the organisms similar to *Methasosarcina* and *Methanoseata* were dominated for all studied influent FA and phenol concentrations.

6. PCR-DGGE technique was used for monitoring dynamic changes of microbial communities in this current study. Changes of both microbial diversities were observed in response to the influent FA and phenol concentrations. Some appearances and losses of bands were visualised and found to be directly related to deterioration and improvement of reactor's performances in removing FA and phenol.

CHAPTER VI

RECOMMENDATIONS FOR FUTURE WORK

The following statements are recommended for future studies.

1. It is interesting to study the possible acclimatisation technique so that higher initial phenol concentration will be efficiently removed at the presence of FA.

2. As previously stated, some bands that appeared on the DGGE gel could be linked to the improvement of FA and phenol removal. It is, therefore, interesting to indentify these microbial species using the suitable molecular technique, e.g. cloning and sequencing.

3. To be able to design and construct the full-scale anaerobic for treating embalming fluid with domestic wastewater, experiments using the pilot-scale reactor will provide some useful information and should be conducted.

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APPENDICES

APPENDIX A

CALIBRATION DATA AND CURVES

Table A-1 Calibration data of VFAs

Method C:\HPCHEM\1\METHODS\NAN1.M

Calibration Table

Determination of acetic acid in water sample

Calib. Data Modified : 9/15/09 4:21:31 PM

Calculate : External Standard
Based on : Peak Area

Rel. Reference Window : 10.000 %
Abs. Reference Window : 0.000 min
Rel. Non-ref. Window : 10.000 %
Abs. Non-ref. Window : 0.000 min
Uncalibrated Peaks : not reported
Partial Calibration : Yes, identified peaks are recalibrated
Correct All Ret. Times: No, only for identified peaks

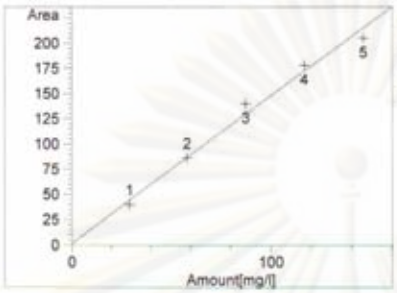
Curve Type : Linear
Origin : Included
Weight : Equal

Recalibration Settings:
Average Response : Average all calibrations
Average Retention Time: Floating Average New 75%

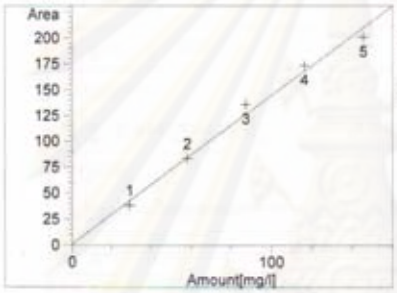
Calibration Report Options :
Printout of recalibrations within a sequence:
Calibration Table after Recalibration
Normal Report after Recalibration
If the sequence is done with bracketing:
Results of first cycle (ending previous bracket)

Signal 1: FID2 B,

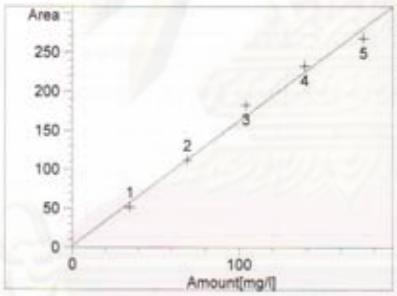
RetTime [min]	Sig	Lvl	Amount [mg/l]	Area	Amt/Area	Ref	Grp	Name
2.262	1	1	20.00000	13.35000	1.49813			Acetic acid
		2	40.00000	29.20189	1.36977			
		3	60.00000	47.57614	1.26114			
		4	80.00000	61.00705	1.31132			
		5	100.00000	71.46004	1.39938			
2.720	1	1	25.46000	27.62615	9.21591e-1			Propionic acid
		2	50.91000	59.90233	8.49883e-1			
		3	76.37000	98.06950	7.78733e-1			
		4	101.83000	125.16159	8.13588e-1			
		5	127.28000	145.47171	8.74947e-1			
2.888	1	1	29.12000	39.83522	7.31011e-1			Isobutyric acid
		2	58.24000	86.31026	6.74775e-1			
		3	87.35000	140.40234	6.22141e-1			
		4	116.47000	178.40808	6.52829e-1			
		5	145.59000	205.37137	7.08911e-1			
3.331	1	1	29.12000	38.45032	7.57341e-1			butyric acid
		2	58.24000	83.39183	6.98390e-1			
		3	87.35000	136.08096	6.41897e-1			
		4	116.47000	173.55798	6.71073e-1			
		5	145.59000	200.90570	7.24668e-1			
3.654	1	1	34.68000	51.89047	6.68331e-1			Isovaleric acid
		2	69.37000	112.27539	6.17856e-1			
		3	104.05000	183.03392	5.68474e-1			
		4	138.74000	232.59976	5.96475e-1			
		5	173.42000	267.68939	6.47840e-1			
4.286	1	1	34.55000	52.58313	6.57055e-1			Valeric acid
		2	69.10000	110.73631	6.24005e-1			
		3	103.65000	180.40026	5.74556e-1			
		4	138.20000	229.46069	6.02282e-1			



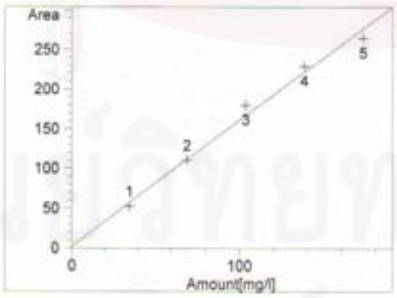
Isobutyric acid at exp. RT: 2.888
FID2 B,
Correlation: 0.99583
Residual Std. Dev.: 8.19102
Formula: y = mx + b
m: 1.46860
b: 1.48128
x: Amount[mg/l]
y: Area



butyric acid at exp. RT: 3.331
FID2 B,
Correlation: 0.99631
Residual Std. Dev.: 7.52712
Formula: y = mx + b
m: 1.43511
b: 9.28870e-1
x: Amount[mg/l]
y: Area

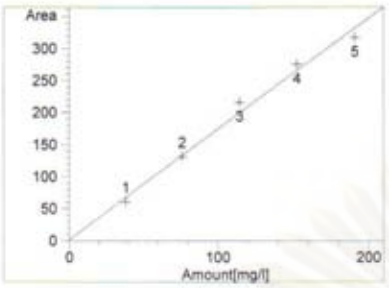


Isovaleric acid at exp. RT: 3.654
FID2 B,
Correlation: 0.99582
Residual Std. Dev.: 10.70039
Formula: y = mx + b
m: 1.60741
b: 1.86940
x: Amount[mg/l]
y: Area

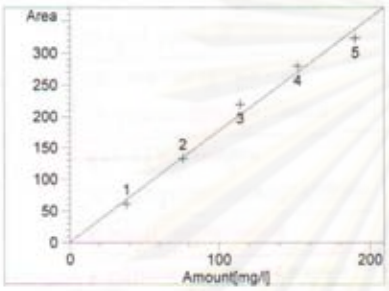


Valeric acid at exp. RT: 4.286
FID2 B,
Correlation: 0.99621
Residual Std. Dev.: 10.05190
Formula: y = mx + b
m: 1.59213
b: 2.17602
x: Amount[mg/l]
y: Area

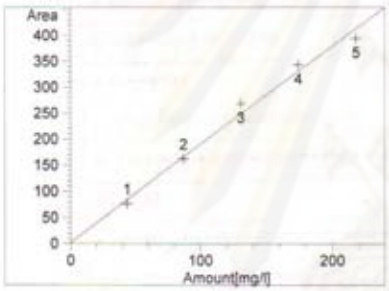
Figure A- 1 Calibration data of VFAs



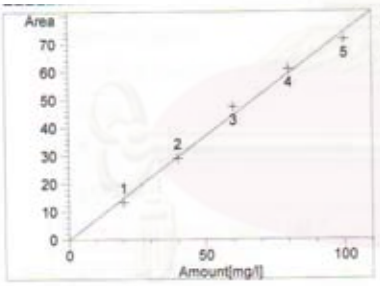
Isocaproic acid at exp. RT: 4.955
FID2 B,
Correlation: 0.99585
Residual Std. Dev.: 12.65097
Formula: y = mx + b
m: 1.73469
b: 1.62285
x: Amount[mg/l]
y: Area



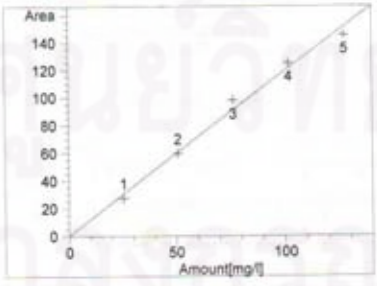
Hexanoic acid at exp. RT: 5.442
FID2 B,
Correlation: 0.99602
Residual Std. Dev.: 12.61854
Formula: y = mx + b
m: 1.77013
b: 1.00484
x: Amount[mg/l]
y: Area



Heptanoic acid at exp. RT: 6.772
FID2 B,
Correlation: 0.99583
Residual Std. Dev.: 15.79610
Formula: y = mx + b
m: 1.89014
b: 1.85758
x: Amount[mg/l]
y: Area



Acetic acid at exp. RT: 2.262
FID2 B,
Correlation: 0.99715
Residual Std. Dev.: 2.34524
Formula: y = mx + b
m: 7.40922e-1
b: 5.30724e-2
x: Amount[mg/l]
y: Area



Propionic acid at exp. RT: 2.720
FID2 B,
Correlation: 0.99657
Residual Std. Dev.: 5.25430
Formula: y = mx + b
m: 1.18762
b: 4.56407e-1
x: Amount[mg/l]
y: Area

Figure A- 2 (cont.) Calibration data of VFAs

APPENDIX B

EXPERIMENTAL DATA

Table B-1 pH and temperature data throughout the operation period

D/M/Y	Time (day)	pH			Temp (˚C)		
		Influent	Reactor A	Reactor B	Influent	Reactor A	Reactor B
16/7/2009	1	6.78	6.92	6.97	25	23	23
17/7/2009	2	6.83	7.07	6.93	25	25	25
18/7/2009	3	6.53	7.01	6.94	25	25	25
19/7/2009	4	6.81	6.99	6.94	25	25	25
20/7/2009	5	6.93	7.01	6.92	25	25	25
21/7/2009	6	6.98	7.32	7.15	25	25	25
22/7/2009	7	6.85	6.98	6.88	24	23	23
23/7/2009	8	6.78	6.95	6.97	25	26	26
24/7/2009	9	6.99	7.35	7.29	25	25	25
25/7/2009	10	7.03	6.94	6.86	25	26	26
26/7/2009	11	6.84	6.9	6.79	25	25	25
27/7/2009	12	6.84	6.91	6.97	25	25	25
28/7/2009	13	7.01	7.11	6.93	25	25	25
29/7/2009	14	6.87	6.85	6.88	25	26	26
30/7/2009	15	6.94	7.24	7.8	25	26	26
31/7/2009	16	6.99	7.21	7.71	25	26	26
1/8/2009	17	6.82	7.04	7.7	25	26	26
2/8/2009	18	6.9	6.83	7.63	25	26	26
3/8/2009	19	6.91	6.85	7.76	25	26	26
4/8/2009	20	7	7.25	7.64	26	26	26
5/8/2009	21	6.86	6.91	6.81	25	25	25
6/8/2009	22	6.83	6.9	6.8	25	25	25
7/8/2009	23	6.74	6.87	6.82	25	25	25
8/8/2009	24	6.83	6.85	6.78	25	25	25
9/8/2009	25	6.79	6.91	6.92	25	25	25
10/8/2009	26	6.74	6.92	6.94	25	25	25
11/8/2009	27	6.79	6.87	6.94	25	25	25
12/8/2009	28	6.74	6.72	6.75	25	25	25
13/8/2009	29	6.78	6.74	6.72	25	25	25
14/8/2009	30	6.07	6.75	6.83	25	25	25
15/8/2009	31	7.01	7.38	7.4	25	25	25
16/8/2009	32	7.04	7.25	7.42	25	25	25
17/8/2009	33	7.15	7.28	7.36	25	25	25
18/8/2009	34	7.08	7.15	7.31	26	27	27
19/8/2009	35	7.2	7.1	7.47	27	28	28
20/8/2009	36	7	7.23	7.27	25	25	25
21/8/2009	37	6.92	7.06	7.22	25	27	27
22/8/2009	38	6.95	7.03	7.03	25	25	25

Table B-1 pH and temperature data throughout the operation period (cont.)

D/M/Y	Time (day)	pH			Temp (˚C)		
		Influent	Reactor A	Reactor B	Influent	Reactor A	Reactor B
23/8/2009	39	6.96	7.08	7.43	26	26	25
24/8/2009	40	7.31	7.28	7.52	25	26	26
25/8/2009	41	7.14	6.85	6.88	27	27	28
26/8/2009	42	7.14	6.65	6.79	25	26	28
27/8/2009	43	7.26	6.85	6.92	24	28	28
28/8/2009	44	7.12	6.85	6.87	26	28	28
29/8/2009	45	7.14	6.8	6.88	26	28	27
30/8/2009	46	7.06	6.74	6.88	26	26	27
31/8/2009	47	7	6.9	6.94	25	27	28
1/9/2009	48	6.98	6.66	6.63	27	28	27
2/9/2009	49	6.91	6.92	6.93	26	27	26
3/9/2009	50	7.02	6.71	6.85	25	26	28
4/9/2009	51	7	7.08	6.82	27	27	28
5/9/2009	52	7.16	6.83	6.85	26	28	27
6/9/2009	53	7	6.7	6.9	25	27	28
7/9/2009	54	7.06	7.06	7	25	25	25
8/9/2009	55	7.06	6.94	7.03	27	29	29
9/9/2009	56	7.06	6.9	6.97	23	25	25
10/9/2009	57	7.1	6.87	7.23	24	25	25
11/9/2009	58	7.09	6.88	6.87	24	25	24
12/9/2009	59	7.15	7.02	6.97	24	25	24
13/9/2009	60	7.17	7.29	7.23	24	24	25
14/9/2009	61	7.16	6.92	6.87	24	24	24
15/9/2009	62	7	6.73	6.92	24	25	24
16/9/2009	63	7.06	6.82	6.85	23	24	25
17/9/2009	64	6.88	6.84	6.73	24	25	24
18/9/2009	65	7.08	6.63	7	24	25	24
19/9/2009	66	7.17	6.87	6.87	24	25	24
20/9/2009	67	7.09	6.92	6.73	24	24	25
21/9/2009	68	7.11	6.75	6.68	24	25	25
22/9/2009	69	7.04	6.69	6.68	24	25	24
23/9/2009	70	7.03	6.46	6.42	24	25	24
24/9/2009	71	6.91	6.63	6.87	24	25	25
25/9/2009	72	7.02	6.87	6.73	23	25	24
26/9/2009	73	7.11	6.59	6.64	24	24	25
27/9/2009	74	6.95	6.73	6.85	24	24	25
28/9/2009	75	6.91	6.82	6.73	24	25	25
29/9/2009	76	6.97	7.09	6.81	23	24	24
30/9/2009	77	6.95	6.75	6.68	24	25	24

Table B-1 pH and temperature data throughout the operation period (cont.)

D/M/Y	Time (day)	pH			Temp (˚C)		
		Influent	Reactor A	Reactor B	Influent	Reactor A	Reactor B
1/10/2009	78	6.85	6.69	6.68	23	25	25
2/10/2009	79	6.97	6.46	6.42	24	25	24
3/10/2009	80	6.97	6.59	6.73	24	24	24
4/10/2009	81	6.96	6.63	6.87	24	24	24
5/10/2009	82	6.89	6.49	6.48	23	25	24
6/10/2009	83	6.85	6.59	6.64	24	24	24
7/10/2009	84	6.87	6.38	6.39	24	24	23
8/10/2009	85	6.95	6.46	6.68	24	24	24
9/10/2009	86	6.85	6.55	6.45	24	24	24
10/10/2009	87	6.96	6.74	6.77	23	24	24
11/10/2009	88	6.89	6.48	6.44	24	23	23
12/10/2009	89	6.85	6.2	6.25	24	24	23
13/10/2009	90	6.85	6.59	6.48	24	24	24
14/10/2009	91	6.87	6.38	6.39	24	24	24
15/10/2009	92	7.1	6.48	6.44	23	23	23
16/10/2009	93	7	6.73	6.92	24	25	24
17/10/2009	94	7.06	6.82	6.85	23	24	25
18/10/2009	95	6.88	6.84	6.73	24	25	24
19/10/2009	96	7.08	6.63	7	24	25	24
20/10/2009	97	7.17	6.87	6.87	24	25	24
21/10/2009	98	7.09	6.92	6.73	24	24	25
22/10/2009	99	7.11	6.75	6.68	24	25	25
23/10/2009	100	7.04	6.69	6.68	24	25	24
24/10/2009	101	7.03	6.46	6.42	24	25	24
25/10/2009	102	6.91	6.63	6.87	24	25	25
26/10/2009	103	7.02	6.87	6.73	23	25	24
27/10/2009	104	7.11	6.59	6.64	24	24	25
28/10/2009	105	6.95	6.73	6.85	24	24	25
29/10/2009	106	6.91	6.82	6.73	24	25	25
30/10/2009	107	6.97	7.09	6.81	23	24	24
31/10/2009	108	6.95	6.75	6.68	24	25	24
1/11/2009	109	6.85	6.69	6.68	23	25	25
2/11/2009	110	6.97	6.46	6.42	24	25	24
3/11/2009	111	6.97	6.59	6.73	24	24	24
4/11/2009	112	6.96	6.63	6.87	24	24	24
5/11/2009	113	6.89	6.49	6.48	23	25	24
6/11/2009	114	6.85	6.59	6.64	24	24	24
7/11/2009	115	6.87	6.38	6.39	24	24	23
8/11/2009	116	6.95	6.46	6.68	24	24	24

Table B-1 pH and temperature data throughout the operation period (cont.)

D/M/Y	Time (day)	pH			Temp (˚C)		
		Influent	Reactor A	Reactor B	Influent	Reactor A	Reactor B
9/11/2009	117	6.85	6.55	6.45	24	24	24
10/11/2009	118	6.96	6.74	6.77	23	24	24
11/11/2009	119	6.89	6.48	6.44	24	23	23
12/11/2009	120	6.85	6.2	6.25	24	24	23
13/11/2009	121	6.85	6.59	6.48	24	24	24
14/11/2009	122	6.87	6.38	6.39	24	24	24
15/11/2009	123	7.1	6.48	6.44	23	23	23
16/11/2009	124	7.11	6.5	6.48	23	23	23
17/11/2009	125	7.2	6.31	6.27	22	22	22
18/11/2009	126	7.01	6.34	6.43	22	22	22
19/11/2009	127	7.01	6.35	6.29	21	21	21
20/11/2009	128	7.05	6.26	6.21	21	21	21
21/11/2009	129	7.04	6.3	6.3	21	21	21
22/11/2009	130	6.97	6.12	6.18	21	21	21
23/11/2009	131	7.09	6.55	6.42	22	22	22
24/11/2009	132	7.11	6.48	6.48	21	21	21
25/11/2009	133	7.04	6.2	6.45	21	21	21
26/11/2009	134	7.03	6.38	6.44	21	21	21
27/11/2009	135	7.02	6.48	6.25	21	21	21
28/11/2009	136	7.05	6.5	6.48	22	22	22
29/11/2009	137	7.04	6.31	6.39	21	21	21
30/11/2009	138	7.07	6.34	6.44	22	22	22
1/12/2009	139	7.09	6.35	6.48	21	21	21
2/12/2009	140	7.04	6.26	6.43	20	20	20
3/12/2009	141	7.05	6.3	6.27	19	19	19
4/12/2009	142	7.07	6.49	6.42	20	20	20
5/12/2009	143	7	6.38	6.21	20	20	20
6/12/2009	144	7.06	6.46	6.44	19	19	19
7/12/2009	145	7.08	6.25	6.46	19	19	19
8/12/2009	146	7.23	6.05	6.13	19	19	19
9/12/2009	147	7.18	6.03	6.01	20	20	20
10/12/2009	148	7.15	6.41	6.37	19	19	19
11/12/2009	149	7.14	6.31	6.24	20	20	20
12/12/2009	150	7.06	6.48	6.48	20	20	20
13/12/2009	151	7	6.38	6.21	19	19	19
14/12/2009	152	7.06	6.46	6.44	19	19	19
15/12/2009	153	7.1	6.25	6.46	19	19	19
16/12/2009	154	7.13	6.44	6.5	20	20	20
17/12/2009	155	7.14	6.47	6.24	20	20	20

Table B-1 pH and temperature data throughout the operation period (cont.)

D/M/Y	Time (day)	pH			Temp (˚C)		
		Influent	Reactor A	Reactor B	Influent	Reactor A	Reactor B
18/12/2009	156	7.14	6.5	6.48	19	19	19
19/12/2009	157	7.18	6.51	6.51	19	19	19
20/12/2009	158	7.12	6.48	6.44	19	19	19
21/12/2009	159	7.14	6.55	6.56	19	19	19
22/12/2009	160	7.26	6.51	6.5	19	19	19
23/12/2009	161	7.2	6.6	6.57	18	18	18
24/12/2009	162	7.16	6.75	6.75	19	19	19
25/12/2009	163	7.21	6.83	6.8	19	19	19
26/12/2009	164	7.25	6.83	6.8	19	19	19
27/12/2009	165	7.29	6.68	6.58	18	18	18
28/12/2009	166	7.2	6.75	6.6	19	19	19
29/12/2009	167	7.21	6.8	6.75	18	18	18
30/12/2009	168	7.25	6.8	6.83	19	19	19
31/12/2009	169	7.2	6.83	6.83	19	19	19
1/1/2010	170	7.01	7	6.63	19	19	19
2/1/2010	171	7.01	6.87	6.87	20	20	20
3/1/2010	172	7.05	6.73	6.59	20	20	20
4/1/2010	173	7.04	6.68	6.73	22	22	22
5/1/2010	174	7.24	6.85	6.82	23	23	23
6/1/2010	175	7.25	6.63	7.09	23	23	23
7/1/2010	176	7.3	6.87	6.75	22	22	22
8/1/2010	177	7.2	6.59	6.64	22	22	22
9/1/2010	178	7.16	6.73	6.85	22	22	22
10/1/2010	179	7.07	6.82	6.73	22	22	22
11/1/2010	180	7.06	7.09	6.75	22	22	22
12/1/2010	181	7.06	6.75	7	22	22	22
13/1/2010	182	7.1	6.64	6.87	22	22	22
14/1/2010	183	7.13	6.85	6.73	22	22	22
15/1/2010	184	7.14	6.73	6.68	22	22	22
16/1/2010	185	7.12	6.65	6.87	21	21	21
17/1/2010	186	7.11	6.68	6.76	21	21	21
18/1/2010	187	7	6.73	6.68	21	21	21
19/1/2010	188	7.09	7.06	6.86	21	21	21
20/1/2010	189	7.07	6.73	6.75	22	22	22
21/1/2010	190	7	6.82	6.64	22	22	22
22/1/2010	191	7.06	7.09	6.85	22	22	22
23/1/2010	192	7.08	6.75	6.73	23	23	23
24/1/2010	193	7.11	6.87	6.75	23	23	23

Table B-1 pH and temperature data throughout the operation period (cont.)

D/M/Y	Time (day)	pH			Temp (° C)		
		Influent	Reactor A	Reactor B	Influent	Reactor A	Reactor B
25/1/2010	194	7.13	6.9	6.79	24	23	23
26/1/2010	195	7.16	7.08	7.01	25	25	25
27/1/2010	196	7.1	6.94	6.99	25	25	25
28/1/2010	197	7.13	7.08	7.02	26	26	26
29/1/2010	198	7.08	7.08	7.05	26	25	25
30/1/2010	199	7.1	7.1	7.02	26	25	26
31/1/2010	200	7.1	6.81	7.08	26	25	25
1/2/2010	201	7.02	6.66	6.82	25	25	24
2/2/2010	202	7	6.92	6.85	27	25	25
3/2/2010	203	7.16	6.71	6.9	26	24	26
4/2/2010	204	7	6.73	6.89	25	25	25
5/2/2010	205	7.06	6.84	6.92	25	24	24
6/2/2010	206	7	6.63	6.87	25	25	25
7/2/2010	207	6.98	6.87	6.88	24	25	25
8/2/2010	208	7.11	6.73	6.88	26	25	26
9/2/2010	209	6.97	6.59	6.75	25	27	27
10/2/2010	210	6.95	6.65	6.39	26	28	28
11/2/2010	211	7.14	6.73	6.99	26	27	27
12/2/2010	212	7.16	6.84	7.02	26	26	26
13/2/2010	213	7.2	7.34	7.43	26	27	26
14/2/2010	214	7.24	7.28	7.25	25	27	28
15/2/2010	215	7.07	6.87	6.87	27	26	27

Table B-2 Gas volume and gas yield data throughout the operation period

D/M/Y	Time (day)	Gas volume(STP)		L CH_4 / gCOD removed	
		Reactor A	Reactor B	Reactor A	Reactor B
16/7/2009	1	0.36	0.36	0.02	0.05
17/7/2009	2	0.36	0.36	0.02	0.05
18/7/2009	3	0.45	0.27	0.03	0.04
19/7/2009	4	0.55	0.36	0.04	0.05
20/7/2009	5	0.54	0.36	0.04	0.05
21/7/2009	6	0.64	0.46	0.03	0.06
22/7/2009	7	0.73	0.45	0.04	0.06
23/7/2009	8	0.90	0.45	0.05	0.06
24/7/2009	9	0.90	0.45	0.06	0.07
25/7/2009	10	1.09	0.91	0.07	0.14
26/7/2009	11	1.18	0.73	0.08	0.12
27/7/2009	12	1.36	0.91	0.10	0.16
28/7/2009	13	1.27	0.91	0.10	0.14
29/7/2009	14	1.18	0.91	0.09	0.14
30/7/2009	15	1.28	1.10	0.10	0.17
31/7/2009	16	1.18	1.18	0.09	0.16
1/8/2009	17	1.36	1.18	0.10	0.16
2/8/2009	18	1.35	1.08	0.10	0.15
3/8/2009	19	1.35	1.08	0.11	0.16
4/8/2009	20	1.44	1.17	0.10	0.18
5/8/2009	21	1.54	1.18	0.10	0.18
6/8/2009	22	1.46	1.09	0.10	0.17
7/8/2009	23	1.55	1.19	0.11	0.18
8/8/2009	24	1.64	1.27	0.11	0.19
9/8/2009	25	1.64	1.37	0.11	0.21
10/8/2009	26	1.62	1.17	0.11	0.17
11/8/2009	27	1.45	1.36	0.10	0.20
12/8/2009	28	1.27	1.09	0.11	0.14
13/8/2009	29	0.27	0.18	0.02	0.02

Table B- 2 Gas volume and gas yield data throughout the operation period (cont.)

D/M/Y	Time (day)	Gas volume(STP)		L CH_4 / gCOD removed	
		Reactor A	Reactor B	Reactor A	Reactor B
14/8/2009	30	0.27	0.18	0.02	0.02
15/8/2009	31	0.36	0.27	0.02	0.04
16/8/2009	32	0.54	0.45	0.03	0.06
17/8/2009	33	0.73	0.46	0.05	0.07
18/8/2009	34	0.82	0.45	0.05	0.06
19/8/2009	35	0.82	0.55	0.05	0.07
20/8/2009	36	0.55	0.36	0.03	0.04
21/8/2009	37	0.45	0.27	0.03	0.04
22/8/2009	38	0.64	0.55	0.05	0.09
23/8/2009	39	0.64	0.46	0.05	0.08
24/8/2009	40	0.72	0.54	0.06	0.08
25/8/2009	41	0.72	0.45	0.06	0.07
26/8/2009	42	0.90	0.45	0.08	0.07
27/8/2009	43	1.08	0.45	0.09	0.07
28/8/2009	44	0.54	0.36	0.04	0.05
29/8/2009	45	0.45	0.45	0.03	0.07
30/8/2009	46	0.54	0.36	0.04	0.05
31/8/2009	47	0.45	0.36	0.04	0.06
1/9/2009	48	0.45	0.45	0.04	0.07
2/9/2009	49	0.63	0.36	0.05	0.06
3/9/2009	50	0.72	0.54	0.06	0.09
4/9/2009	51	0.45	0.27	0.05	0.06
5/9/2009	52	0.45	0.27	0.05	0.06
6/9/2009	53	0.45	0.27	0.05	0.06
7/9/2009	54	0.45	0.27	0.05	0.05
8/9/2009	55	0.45	0.27	0.05	0.05
9/9/2009	56	0.63	0.54	0.05	0.08
10/9/2009	57	0.63	0.45	0.05	0.07
11/9/2009	58	0.63	0.54	0.05	0.08
12/9/2009	59	0.54	0.45	0.05	0.07
13/9/2009	60	0.63	0.45	0.05	0.07

Table B- 2 Gas volume and gas yield data throughout the operation period (cont.)

D/M/Y	Time (day)	Gas volume(STP)		L CH_4 / gCOD removed	
		Reactor A	Reactor B	Reactor A	Reactor B
14/9/2009	61	0.63	0.45	0.05	0.07
15/9/2009	62	0.64	0.54	0.03	0.05
16/9/2009	63	0.63	0.45	0.03	0.04
17/9/2009	64	0.64	0.55	0.03	0.05
18/9/2009	65	0.91	0.73	0.03	0.06
19/9/2009	66	1.00	0.73	0.04	0.06
20/9/2009	67	1.19	1.09	0.05	0.09
21/9/2009	68	1.27	1.27	0.05	0.11
22/9/2009	69	1.18	1.09	0.05	0.10
23/9/2009	70	1.45	1.18	0.06	0.10
24/9/2009	71	1.26	1.26	0.05	0.11
25/9/2009	72	1.27	1.09	0.06	0.10
26/9/2009	73	1.28	1.28	0.06	0.12
27/9/2009	74	1.28	1.09	0.06	0.10
28/9/2009	75	1.37	0.91	0.06	0.08
29/9/2009	76	1.18	1.09	0.05	0.10
30/9/2009	77	1.27	1.27	0.06	0.11
1/10/2009	78	1.27	1.09	0.06	0.10
2/10/2009	79	1.27	1.09	0.06	0.10
3/10/2009	80	1.19	1.10	0.05	0.10
4/10/2009	81	1.27	1.27	0.06	0.11
5/10/2009	82	1.36	1.18	0.05	0.11
6/10/2009	83	0.91	0.91	0.04	0.08
7/10/2009	84	0.72	0.82	0.03	0.08
8/10/2009	85	1.09	0.90	0.04	0.07
9/10/2009	86	1.08	0.90	0.04	0.07
10/10/2009	87	1.08	1.08	0.04	0.08
11/10/2009	88	1.27	1.18	0.05	0.09
12/10/2009	89	1.09	1.00	0.05	0.08
13/10/2009	90	1.09	1.00	0.05	0.07
14/10/2009	91	1.09	1.00	0.05	0.07

Table B- 2 Gas volume and gas yield data throughout the operation period (cont.)

D/M/Y	Time (day)	Gas volume(STP)		L CH_4 / gCOD removed	
		Reactor A	Reactor B	Reactor A	Reactor B
15/10/2009	92	1.09	1.00	0.05	0.07
16/10/2009	93	1.18	1.00	0.05	0.07
17/10/2009	94	1.18	1.09	0.05	0.08
18/10/2009	95	1.18	1.09	0.05	0.08
19/10/2009	96	1.36	1.18	0.06	0.09
20/10/2009	97	1.82	1.18	0.08	0.09
21/10/2009	98	1.82	1.18	0.08	0.09
22/10/2009	99	1.91	1.18	0.08	0.09
23/10/2009	100	2.27	1.36	0.10	0.10
24/10/2009	101	1.91	1.18	0.09	0.09
25/10/2009	102	2.28	1.46	0.11	0.11
26/10/2009	103	1.92	1.28	0.09	0.10
27/10/2009	104	1.84	1.19	0.09	0.09
28/10/2009	105	1.84	1.19	0.09	0.09
29/10/2009	106	1.83	1.19	0.09	0.09
30/10/2009	107	1.93	1.29	0.09	0.10
31/10/2009	108	1.92	1.19	0.09	0.09
1/11/2009	109	1.01	1.19	0.10	0.12
2/11/2009	110	1.10	1.10	0.10	0.11
3/11/2009	111	1.19	1.10	0.12	0.11
4/11/2009	112	1.29	1.10	0.13	0.11
5/11/2009	113	1.28	1.19	0.13	0.12
6/11/2009	114	1.47	1.29	0.07	0.10
7/11/2009	115	1.47	1.47	0.07	0.11
8/11/2009	116	1.65	1.47	0.08	0.11
9/11/2009	117	1.38	1.38	0.07	0.11
10/11/2009	118	1.19	1.10	0.09	0.11
11/11/2009	119	1.29	1.20	0.10	0.12
12/11/2009	120	1.10	1.10	0.09	0.11
13/11/2009	121	1.65	1.47	0.07	0.10
14/11/2009	122	1.75	1.38	0.08	0.09
15/11/2009	123	1.84	1.38	0.08	0.09
16/11/2009	124	1.84	1.38	0.07	0.08
17/11/2009	125	1.85	1.39	0.07	0.08
18/11/2009	126	2.13	1.30	0.08	0.07

Table B- 2 Gas volume and gas yield data throughout the operation period (cont.)

D/M/Y	Time (day)	Gas volume(STP)		L CH_4 / gCOD removed	
		Reactor A	Reactor B	Reactor A	Reactor B
19/11/2009	127	2.14	1.39	0.08	0.08
20/11/2009	128	2.14	1.39	0.08	0.08
21/11/2009	129	2.04	1.30	0.08	0.08
22/11/2009	130	2.04	1.49	0.08	0.09
23/11/2009	131	2.04	1.39	0.08	0.09
24/11/2009	132	2.04	1.39	0.08	0.09
25/11/2009	133	2.04	1.39	0.08	0.09
26/11/2009	134	2.04	1.39	0.08	0.09
27/11/2009	135	2.04	1.39	0.08	0.09
28/11/2009	136	2.04	1.39	0.08	0.09
29/11/2009	137	2.04	1.39	0.08	0.09
30/11/2009	138	2.04	1.39	0.08	0.09
1/12/2009	139	2.04	1.39	0.04	0.04
2/12/2009	140	2.05	1.49	0.04	0.05
3/12/2009	141	2.06	1.59	0.04	0.05
4/12/2009	142	2.05	1.86	0.04	0.07
5/12/2009	143	2.61	2.05	0.05	0.07
6/12/2009	144	2.80	3.46	0.05	0.12
7/12/2009	145	2.52	3.37	0.05	0.12
8/12/2009	146	2.62	3.18	0.05	0.10
9/12/2009	147	2.98	2.80	0.06	0.09
10/12/2009	148	2.80	3.93	0.06	0.13
11/12/2009	149	2.80	3.54	0.06	0.14
12/12/2009	150	2.80	3.73	0.06	0.15
13/12/2009	151	2.80	3.74	0.06	0.15
14/12/2009	152	2.62	3.74	0.06	0.15
15/12/2009	153	2.52	3.74	0.05	0.12
16/12/2009	154	2.52	4.47	0.05	0.14
17/12/2009	155	2.42	4.66	0.04	0.15
18/12/2009	156	2.80	4.67	0.06	0.15
19/12/2009	157	3.83	4.67	0.08	0.15
20/12/2009	158	3.83	4.67	0.08	0.15
21/12/2009	159	4.86	4.67	0.10	0.15
22/12/2009	160	4.67	4.49	0.10	0.15
23/12/2009	161	4.69	4.22	0.10	0.14
24/12/2009	162	4.67	4.21	0.10	0.14
25/12/2009	163	4.77	4.67	0.10	0.16
26/12/2009	164	4.67	4.67	0.10	0.16

Table B-2 Gas volume and gas yield data throughout the operation period (cont.)

D/M/Y	Time (day)	Gas volume(STP)		L CH_4 / gCOD removed	
		Reactor A	Reactor B	Reactor A	Reactor B
27/12/2009	165	4.50	4.50	0.09	0.15
28/12/2009	166	4.49	4.86	0.10	0.17
29/12/2009	167	4.32	4.69	0.09	0.16
30/12/2009	168	4.21	4.77	0.09	0.17
31/12/2009	169	4.21	4.77	0.09	0.17
1/1/2010	170	4.30	4.67	0.09	0.16
2/1/2010	171	1.30	2.80	0.11	0.24
3/1/2010	172	1.40	2.33	0.12	0.20
4/1/2010	173	1.39	2.04	0.11	0.17
5/1/2010	174	1.11	1.66	0.06	0.13
6/1/2010	175	1.11	1.84	0.06	0.15
7/1/2010	176	1.02	1.85	0.05	0.15
8/1/2010	177	0.93	1.85	0.05	0.15
9/1/2010	178	1.39	1.85	0.07	0.15
10/1/2010	179	0.93	1.85	0.05	0.15
11/1/2010	180	0.93	1.85	0.04	0.12
12/1/2010	181	0.93	1.85	0.04	0.13
13/1/2010	182	0.93	1.85	0.04	0.13
14/1/2010	183	0.93	1.85	0.04	0.13
15/1/2010	184	0.93	1.85	0.04	0.13
16/1/2010	185	0.93	1.86	0.04	0.13
17/1/2010	186	0.93	1.67	0.04	0.11
18/1/2010	187	0.93	1.86	0.04	0.13
19/1/2010	188	1.11	2.04	0.05	0.12
20/1/2010	189	0.93	1.94	0.04	0.12
21/1/2010	190	1.11	1.85	0.05	0.11
22/1/2010	191	0.93	1.85	0.04	0.11
23/1/2010	192	1.01	1.84	0.04	0.11
24/1/2010	193	1.11	1.84	0.05	0.11
25/1/2010	194	1.01	1.84	0.04	0.12
26/1/2010	195	0.82	2.02	0.04	0.13
27/1/2010	196	1.37	2.02	0.06	0.13
28/1/2010	197	0.91	2.28	0.04	0.15
29/1/2010	198	0.46	1.83	0.02	0.12
30/1/2010	199	0.46	1.37	0.02	0.09
31/1/2010	200	0.27	1.37	0.01	0.09
1/2/2010	201	0.18	1.37	0.01	0.08
2/2/2010	202	0.18	1.37	0.01	0.08

Table B-2 Gas volume and gas yield data throughout the operation period (cont.)

D/M/Y	Time (day)	Gas volume(STP)		L CH_4 / gCOD removed	
		Reactor A	Reactor B	Reactor A	Reactor B
3/2/2010	203	0.18	1.37	0.01	0.08
4/2/2010	204	0.18	1.83	0.01	0.10
5/2/2010	205	0.18	1.65	0.01	0.09
6/2/2010	206	0.27	1.74	0.01	0.10
7/2/2010	207	0.18	1.65	0.01	0.09
8/2/2010	208	0.27	1.64	0.01	0.10
9/2/2010	209	0.46	1.83	0.02	0.11
10/2/2010	210	0.91	2.28	0.04	0.14
11/2/2010	211	0.91	2.19	0.04	0.13
12/2/2010	212	0.91	2.10	0.04	0.13
13/2/2010	213	0.91	2.01	0.04	0.12
14/2/2010	214	0.92	2.20	0.04	0.13
15/2/2010	215	0.91	2.28	0.03	0.14

Table B- 3 Alkalinity and VFA data during the start up period

D/M/Y	Time (day)	Influent (Alk)	Effluent (Alk)		Influent (VFA)	Effluent (VFA)		Influent (VFA/Alk)	Effluent (VFA/alk)	
			6 h	12 h		6 h	12h		6 h	12 h
17/7/2009	2	200	198	176	42	48	58	0.21	0.24	0.33
21/7/2009	6	168	200	198	48	46	40	0.29	0.23	0.20
24/7/2009	9	152	170	178	40	46	42	0.26	0.27	0.24
28/7/2009	13	150	168	174	38	38	42	0.25	0.23	0.24
31/7/2009	16	140	182	184	40	42	40	0.29	0.23	0.22
4/8/2009	20	158	192	178	50	58	48	0.32	0.30	0.27
7/8/2009	23	154	168	182	64	52	60	0.42	0.31	0.33
10/8/2009	26	144	162	178	66	56	64	0.46	0.35	0.36
11/8/2009	27	144	156	178	60	50	62	0.42	0.32	0.35

Table B-4 SS data throughout the operation period

D/M/Y	Time (day)	Influent	Effluent		% Removed	
			6 h	12 h	6 h	12 h
17/7/2009	2	118	18	8	85	93
21/7/2009	6	100	20	17	80	83
24/7/2009	9	93	24	4	74	95
28/7/2009	13	84	10	8	88	91
31/7/2009	16	124	15	4	88	97
4/8/2009	20	117	9	4	92	97
7/8/2009	23	109	16	6	85	94
10/8/2009	26	94	22	12	77	88
11/8/2009	27	94	21	15	78	85
14/8/2009	30	89	23	14	74	85
15/8/2009	31	94	25	6	73	94
18/8/2009	34	75	18	2	76	97
21/8/2009	37	83	8	6	91	93
25/8/2009	41	23	16	11	31	54
28/8/2009	44	84	21	5	75	94
1/9/2009	48	72	5	4	92	94
4/9/2009	51	174	15	6	91	97
9/9/2009	56	158	148	7	7	96
11/9/2009	58	101	26	7	74	93
13/9/2009	60	84	56	16	34	81
15/9/2009	62	64	14	17	79	73
18/9/2009	65	52	33	10	37	81
22/9/2009	69	40	21	12	48	70
25/9/2009	72	41	21	11	49	72
29/9/2009	76	33	21	6	36	81
1/10/2009	78	38	23	10	40	75
3/10/2009	80	48	32	7	34	86
5/10/2009	82	32	16	3	50	90
9/10/2009	86	52	5	6	91	89
13/10/2009	90	56	4	7	93	87
16/10/2009	93	84	19	12	78	86
20/10/2009	97	38	12	16	69	59
23/10/2009	100	52	14	21	73	59
26/10/2009	103	40	21	9	47	77
28/10/2009	105	56	27	31	52	44
30/10/2009	107	49	23	24	53	52
2/11/2009	110	74	21	30	72	60
6/11/2009	114	59	25	25	57	57
10/11/2009	118	68	15	12	78	82
13/11/2009	121	52	25	10	52	80
16/11/2009	124	37	33	23	11	37
19/11/2009	127	39	28	2	28	95
24/11/2009	132	39	29	22	26	44
27/11/2009	135	39	30	19	24	52
30/11/2009	138	33	20	2	40	94

Table B-4 SS data throughout the operation period (cont.)

D/M/Y	Time (day)	Influent	Effluent		% Removed	
			6 h	12 h	6 h	12 h
1/12/2009	139	39	30	29	24	27
4/12/2009	142	61	31	32	50	47
8/12/2009	146	70	17	25	75	64
11/12/2009	149	117	18	14	85	88
15/12/2009	153	74	8	17	90	77
18/12/2009	156	72	16	14	78	81
22/12/2009	160	73	21	20	71	73
25/12/2009	163	69	5	8	92	88
28/12/2009	166	91	40	36	57	61
30/12/2009	168	80	59	25	27	69
1/1/2010	170	87	65	31	26	64
4/1/2010	173	76	15	8	80	90
11/1/2010	180	158	128	1	19	99
18/1/2010	187	138	26	36	81	74
25/1/2010	194	131	56	25	58	81
1/2/2010	201	112	14	12	88	89
8/2/2010	208	101	33	19	67	81
15/2/2010	215	95	21	11	78	89

Table B-5 VSS data throughout the operation period

D/M/Y	Time (day)	Influent	Effluent		% Removed	
			6 h	12 h	6 h	12 h
17/7/2009	2	89	17	7	81	92
21/7/2009	6	92	15	10	83	89
24/7/2009	9	86	22	1	74	99
28/7/2009	13	69	10	6	86	91
31/7/2009	16	109	7	4	94	97
4/8/2009	20	91	3	3	96	97
7/8/2009	23	87	14	4	84	96
10/8/2009	26	84	7	7	91	91
11/8/2009	27	84	12	8	86	90
14/8/2009	30	77	18	12	76	85
15/8/2009	31	83	17	5	80	94
18/8/2009	34	66	17	0	75	100
21/8/2009	37	75	5	1	94	99
25/8/2009	41	22	15	8	33	65
28/8/2009	44	70	16	4	77	94
1/9/2009	48	48	1	4	97	91
4/9/2009	51	119	6	6	95	95
9/9/2009	56	104	7	7	93	93
11/9/2009	58	57	6	6	89	89
13/9/2009	60	60	7	8	88	86
15/9/2009	62	46	30	15	35	68
18/9/2009	65	47	20	5	58	90
22/9/2009	69	39	10	7	75	81

Table B-5 VSS data throughout the operation period (cont)

D/M/Y	Time (day)	Influent	Effluent		% Removed	
			6 h	12 h	6 h	12 h
25/9/2009	72	40	10	7	76	83
29/9/2009	76	30	4	4	85	85
1/10/2009	78	34	2	2	95	95
3/10/2009	80	34	5	5	86	86
5/10/2009	82	12	3	2	74	82
9/10/2009	86	11	10	1	10	87
13/10/2009	90	39	7	1	83	98
16/10/2009	93	40	7	7	81	83
20/10/2009	97	60	30	8	50	86
23/10/2009	100	36	2	30	95	16
26/10/2009	103	41	30	2	27	96
28/10/2009	105	36	18	30	48	16
30/10/2009	107	56	12	14	78	76
2/11/2009	110	46	2	22	95	51
6/11/2009	114	63	21	21	67	67
10/11/2009	118	56	23	2	59	97
13/11/2009	121	54	12	9	77	83
16/11/2009	124	45	2	34	96	25
19/11/2009	127	30	2	2	93	95
24/11/2009	132	11	1	5	89	61
27/11/2009	135	40	17	6	58	85
30/11/2009	138	36	19.1	7.4	47	79
1/12/2009	139	36	25	30	29	16
4/12/2009	142	30	4	25	86	15
8/12/2009	146	34	13	25	63	27
11/12/2009	149	39	17	13	56	65
15/12/2009	153	51	18	16	65	69
18/12/2009	156	68	17	13	75	80
22/12/2009	160	57	8	19	85	67
25/12/2009	163	67	5	3	92	95
4/1/2010	173	45	8	3	82	92
11/1/2010	180	46	31	1	32	98
18/1/2010	187	43	37	31	14	28
25/1/2010	194	80	47	31	41	61
1/2/2010	201	63	45	14	28	78
8/2/2010	208	80	22	5	72	93
15/2/2010	215	73	20	7	72	91

Table B-6 COD and FCOD data throughout the operation period

D/M/Y	Time (day)	Influent	Effluent		COD Remove		% Removed	
			6 h	12 h	6 h	12h	6 h	12 h
17/7/2009	2	169	23	11	146	158	87	93
21/7/2009	6	203	41	26	162	177	80	87
24/7/2009	9	178	35	31	143	147	80	83
28/7/2009	13	184	38	23	147	162	80	88
31/7/2009	16	202	53	20	150	182	74	90
4/8/2009	20	207	36	32	171	175	83	85
7/8/2009	23	209	43	35	167	174	80	83
10/8/2009	26	234	50	36	173	177	78	82
11/8/2009	27	234	53	36	141	198	80	84
14/8/2009	30	268	55	39	213	228	79	85
15/8/2009	31	250	48	48	202	202	81	81
18/8/2009	34	254	50	15	204	238	80	94
21/8/2009	37	203	41	26	162	177	80	87
25/8/2009	41	179	31	19	149	160	83	89
28/8/2009	44	196	15	15	181	181	92	92
1/9/2009	48	215	45	24	170	190	79	89
4/9/2009	51	166	36	20	130	146	78	88
9/9/2009	56	214	47	19	167	195	78	91
11/9/2009	58	228	44	20	184	208	81	91
13/9/2009	60	212	32	20	180	192	85	91
15/9/2009	62	394	96	60	299	335	76	85
18/9/2009	65	386	36	32	351	355	91	92
22/9/2009	69	394	39	35	354	358	90	91
25/9/2009	72	398	67	55	331	343	83	86
29/9/2009	76	375	44	28	331	347	88	93
1/10/2009	78	399	48	28	351	371	88	93
3/10/2009	80	405	69	36	336	368	83	91
5/10/2009	82	400	52	24	348	376	87	94
9/10/2009	86	470	40	28	431	443	92	94
13/10/2009	90	478	126	27	352	451	74	94
16/10/2009	93	471	128	23	342	447	73	95
20/10/2009	97	467	121	27	346	440	74	94
23/10/2009	100	477	136	20	341	457	72	96
26/10/2009	103	474	109	24	364	450	77	95
28/10/2009	105	484	135	23	349	461	72	95
30/10/2009	107	482	115	16	368	466	76	97
2/11/2009	110	667	500	315	167	352	25	53
6/11/2009	114	709	358	264	350	445	49	63
10/11/2009	118	697	390	261	307	436	44	63
13/11/2009	121	712	394	246	318	466	45	65
16/11/2009	124	912	440	264	472	648	52	71
19/11/2009	127	882	403	278	479	604	54	68
24/11/2009	132	791	369	266	422	525	53	66
27/11/2009	135	817	354	300	463	518	57	63

Table B-6 COD and FCOD data throughout the operation period (cont.)

D/M/Y	Time (day)	Influent	Effluent		COD Remove		% Removed	
			6 h	12 h	6 h	12h	6 h	12 h
30/11/2009	138	818	409	295	409	523	50	64
1/12/2009	139	1828	871	657	956	1171	52	64
4/12/2009	142	1829	853	766	976	1063	53	58
8/12/2009	146	1882	894	685	988	1197	53	64
11/12/2009	149	1686	798	717	888	969	53	57
15/12/2009	153	1853	809	644	1044	1208	56	65
18/12/2009	156	1813	840	624	973	1189	54	66
22/12/2009	160	1720	812	608	908	1112	53	65
25/12/2009	163	1706	790	591	917	1115	54	65
28/12/2009	166	1687	786	512	901	1175	53	70
30/12/2009	168	1720	778	530	943	1190	55	69
1/1/2010	170	1694	763	538	932	1156	55	68
4/1/2010	173	728	504	280	224	448	31	62
11/1/2010	180	751	392	275	359	476	48	63
18/1/2010	187	762	361	295	400	467	53	61
25/1/2010	194	767	395	245	372	523	49	68
1/2/2010	201	749	401	148	348	601	46	80
8/2/2010	208	686	277	77	409	609	60	89
15/2/2010	215	684	234	75	450	609	66	89

Table B-7 Gas compositions data throughout the experimental period

D/M/Y	Time (day)	Reactor A				Reactor B			
		O_2	CO_2	CH_4	N_2	O_2	CO_2	CH_5	N_3
26/7/2009	11		62.3	37.7		5	49.4	45.6	
2/8/2009	18	5	49.8	45.1		5.1	45.1	49.8	
9/8/2009	25	8.3	43.6	46.5	1.6	6.3	40.5	53.2	
16/8/2009	32	7.3	42.1	48.9	1.7	3.6	44.3	52.1	
23/8/2009	39	5.5	42.5	48.5	3.5	4	40.9	55	
30/8/2009	46	5.4	44.7	49.9		5.2	34.3	58.5	2
6/9/2009	53		48.5	51.5		7.1	36.8	52.7	3.4
13/9/2009	60		39.8	57.2	3.1	5.5	30.3	59.6	5.6
20/9/2009	67	6.2	32.8	58	3	4.7	33.6	56.9	4.8
27/9/2009	74	6.2	40	53.7			38	61	1
4/10/2009	81	3.7	34.1	58.9	3.3	3.5	30.1	63.1	3.2
11/10/2009	88	5	31	58.9	5	3.9	30.1	62	4
18/10/2009	95	6.3	40.5	53.2		3.3	31.8	64.9	
25/10/2009	102	5	32	62.5		4	30.6	65.4	
1/11/2009	109	5	31.6	63.4		3.6	28.1	68.4	
8/11/2009	116	3.9	25.6	68.5	2	3.8	26	68.6	1.6
15/11/2009	123	3.5	28.1	68.4		3.2	23.5	71.1	2.2

Table B-7 Gas compositions data throughout the experimental period (cont.)

D/M/Y	Time (day)	Reactor A				Reactor B			
		O_2	CO_2	CH_4	N_2	O_2	CO_2	CH_5	N_3
22/11/2009	130	1.7	19.3	73.3	5.7	5.1	20	70.8	4.2
29/11/2009	137	2.5	20.1	73.1	4.2	1.9	20.3	74.7	3
6/12/2009	144	3.8	18.1	74.7	3.4	2.6	19.5	75	2.9
13/12/2009	151	2.5	15.1	79.1	3.3	7	13.1	76.8	3.1
20/12/2009	158	5.5	14	76.5	4	5	10.8	77.6	6.6
27/12/2009	165	2.3	13.7	74.7	9.2	2.4	14.8	77.3	7.4
3/1/2010	172	7	15.7	77.8	4.2	2.7	15.9	77.3	4.1
10/1/2010	179	2.6	22	72.8	2.6	2.4	16.5	76.5	4.6
17/1/2010	186	2.3	29.2	63.2	5.3	2.9	29	63.8	4.3
24/1/2010	193	2.3	30.4	61.9	5.4	3.4	29.2	63.2	4.2
31/1/2010	200	3.6	25.4	65.9	5.1	2.4	26.4	66.5	4.6
7/2/2010	207	4.7	25.9	67.3	2.1	3.9	20.7	68.8	6.6
14/2/2010	214	4	25.9	67.3	2.8	2.3	29.7	72.8	5.3

Table B-8 FA data throughout the operation period

D/M/Y	Time (day)	Inlet	Outlet		% Removed	
			6 h	12 h	6 h	12 h
14/8/2009	30	16.71	1.8	1.1	89.3	93.6
18/8/2009	34	14.63	2.9	0.6	80.2	96.0
21/8/2009	37	16.11	2.2	2.1	86.3	87.1
25/8/2009	41	17.17	2.0	2.0	88.4	88.2
28/8/2009	44	21.79	1.0	0.9	95.5	95.7
1/9/2009	48	22.65	1.2	1.0	94.9	95.6
4/9/2009	51	22.30	0.48	0.44	97.8	98.0
9/9/2009	56	21.94	0.36	0.36	98.4	98.4
11/9/2009	58	21.69	0.36	0.36	98.3	98.3
13/9/2009	60	23.33	0.35	0.39	98.5	98.3
15/9/2009	62	58.83	3.88	0.83	93.4	98.6
18/9/2009	65	63.97	0.35	0.44	99.5	99.3
22/9/2009	69	58.80	0.89	0.68	98.5	98.9
25/9/2009	72	63.31	0.28	0.27	99.6	99.6
29/9/2009	76	59.99	0.49	0.45	99.2	99.2
1/10/2009	78	65.88	0.43	0.29	99.4	99.6
3/10/2009	80	65.06	0.39	0.26	99.4	99.6
5/10/2009	82	64.45	0.63	0.50	99.0	99.2
9/10/2009	86	129.25	0.68	0.55	99.5	99.6
13/10/2009	90	131.48	0.77	0.59	99.4	99.5

Table B-8 FA data throughout the operation period (cont.)

D/M/Y	Time (day)	Inlet	Outlet		% Removed	
			6 h	12 h	6 h	12 h
16/10/2009	93	132.82	0.72	0.61	99.5	99.5
20/10/2009	97	130.50	0.39	0.26	99.7	99.8
23/10/2009	100	126.76	0.35	0.27	99.7	99.8
26/10/2009	103	116.19	0.40	0.32	99.7	99.7
28/10/2009	105	131.76	0.43	0.29	99.7	99.8
30/10/2009	107	130.13	0.32	0.26	99.8	99.8
2/11/2009	110	405.35	14.29	3.01	96.5	99.3
6/11/2009	114	403.47	13.11	1.18	96.8	99.7
10/11/2009	118	399.73	13.01	1.29	96.7	99.7
13/11/2009	121	399.92	12.84	1.34	96.8	99.7
16/11/2009	124	412.83	13.93	1.32	96.6	99.7
19/11/2009	127	411.89	13.93	1.21	96.6	99.7
24/11/2009	132	405.35	13.70	1.24	96.6	99.7
27/11/2009	135	410.02	14.02	1.17	96.6	99.7
30/11/2009	138	409.65	12.84	1.24	96.9	99.7
1/12/2009	139	1448.18	14.35	1.26	99.0	99.9
4/12/2009	142	1467.83	14.93	1.26	99.0	99.9
8/12/2009	146	1464.55	14.00	1.93	99.0	99.9
11/12/2009	149	1402.34	14.02	1.69	99.0	99.9
15/12/2009	153	1408.88	14.42	1.38	99.0	99.9
18/12/2009	156	1408.88	14.35	1.92	99.0	99.9
22/12/2009	160	1346.01	14.79	1.55	98.9	99.9
25/12/2009	163	1356.49	14.93	1.75	98.9	99.9
28/12/2009	166	1346.01	15.01	1.57	98.9	99.9
30/12/2009	168	1356.49	13.70	1.04	99.0	99.9
1/1/2010	170	1399.72	13.49	1.63	99.0	99.9
4/1/2010	173	385.83	1.74	1.16	99.5	99.7
11/1/2010	180	387.47	1.77	1.10	99.5	99.7
18/1/2010	187	407.77	1.57	0.64	99.6	99.8
25/1/2010	194	418.91	1.01	0.92	99.8	99.8
1/2/2010	201	410.39	0.98	0.95	99.8	99.8
8/2/2010	208	385.84	0.21	0.20	99.9	99.9
15/2/2010	215	375.51	0.29	0.15	99.9	100.0

Table B-9 Phenol data throughout the operation period

D/M/Y	Time (day)	Inlet	Outlet		% Removed	
			6 h	12 h	6 h	12 h
14/8/2009	30	3.00	1.80	0.94	39.90	68.75
18/8/2009	34	3.14	2.03	0.72	35.32	77.04
21/8/2009	37	3.84	0.84	0.16	78.10	95.91
25/8/2009	41	3.39	0.16	0.11	95.37	96.63
28/8/2009	44	5.14	0.19	0.18	96.32	96.53
1/9/2009	48	6.08	1.21	0.48	80.08	92.14
4/9/2009	51	5.79	1.96	0.38	66.09	93.40
9/9/2009	56	6.44	1.07	0.44	83.31	93.14
11/9/2009	58	6.35	1.00	0.41	84.19	93.61
13/9/2009	60	6.08	1.11	0.39	81.81	93.62
15/9/2009	62	13.46	4.39	0.76	67.39	94.32
18/9/2009	65	15.72	5.02	0.70	68.08	95.55
22/9/2009	69	15.49	7.26	0.21	53.14	98.67
25/9/2009	72	16.64	5.45	0.11	67.22	99.33
29/9/2009	76	13.05	3.92	0.17	69.95	98.69
1/10/2009	78	16.07	5.58	0.10	65.26	99.36
3/10/2009	80	14.63	4.35	0.24	70.27	98.35
5/10/2009	82	15.79	5.60	0.10	64.52	99.35
9/10/2009	86	30.88	22.34	0.12	27.65	99.62
13/10/2009	90	31.63	22.48	0.10	28.92	99.70
16/10/2009	93	33.16	23.22	2.59	29.96	92.19
20/10/2009	97	35.10	22.96	0.69	34.59	98.03
23/10/2009	100	33.02	22.85	0.59	30.80	98.22
26/10/2009	103	32.81	21.66	0.69	33.98	97.89
28/10/2009	105	32.81	21.66	0.69	33.98	97.89
30/10/2009	107	30.34	19.58	0.40	35.47	98.67
2/11/2009	110	104.90	70.26	35.87	33.02	65.81
6/11/2009	114	93.80	63.56	32.14	32.24	65.74
10/11/2009	118	92.47	61.73	29.81	33.24	67.77
13/11/2009	121	95.75	61.42	30.78	35.85	67.85
16/11/2009	124	83.22	58.30	25.34	29.95	69.56
19/11/2009	127	80.98	58.36	26.26	27.93	67.57
24/11/2009	132	81.32	57.47	27.09	29.33	66.69
27/11/2009	135	79.21	57.57	27.24	27.31	65.60
30/11/2009	138	82.77	59.54	27.11	28.07	67.25
1/12/2009	139	217.62	203.03	190.39	6.70	12.51
4/12/2009	142	217.41	198.66	186.99	8.62	13.99

Table B-9 Phenol data throughout the operation period (cont.**)**

D/M/Y	Time (day)	Inlet	Outlet		% Removed	
			6 h	12 h	6 h	12 h
8/12/2009	146	202.70	184.63	173.67	8.91	14.32
11/12/2009	149	212.73	193.49	182.17	9.04	14.37
15/12/2009	153	218.71	199.05	186.65	8.99	14.66
18/12/2009	156	214.97	196.48	180.88	8.60	15.86
22/12/2009	160	212.73	194.56	180.24	8.54	15.27
25/12/2009	163	199.26	182.38	168.27	8.47	15.55
28/12/2009	166	218.50	200.01	185.23	8.46	15.22
30/12/2009	168	218.50	199.48	186.76	8.71	14.53
1/1/2010	170	190.93	174.48	163.14	8.62	14.55
4/1/2010	173	83.18	58.28	54.54	29.93	34.43
11/1/2010	180	81.14	55.87	54.48	31.14	32.86
18/1/2010	187	77.03	55.93	55.07	27.40	28.51
25/1/2010	194	84.14	57.21	56.14	32.01	33.28
1/2/2010	201	76.68	60.52	48.41	21.07	36.87
8/2/2010	208	76.89	54.48	36.92	29.15	51.98
15/2/2010	215	72.91	48.11	12.96	34.01	82.22

Table B-10 Influent VFAs composition data throughout the operation period

D/M/Y	Time (day)	Influent								
		Ac	Pr	Isobu	Bu	Isova	Va	Isoca	Ca	Hep
17/7/2009	2	3.26				1.11			0.69	
21/7/2009	6	2.39							2.02	
24/7/2009	9	4.04				1.92			0.99	
28/7/2009	13	3.42				0.5			0.47	
31/7/2009	16	6.74								
4/8/2009	20	3.12							0.63	
7/8/2009	23	5.24							0.5	
10/8/2009	26	5.31							0.17	
11/8/2009	27	5.11							0.16	
14/8/2009	30	8.11	1.65							
18/8/2009	34	4.3	0.98							
21/8/2009	37	2.84	0.74							
25/8/2009	41	5.16				1.51			0.54	
28/8/2009	44	4.53				2.33			2.9	
1/9/2009	48	3.74	0.74							0.79
4/9/2009	51	5	1.24		1.07	5.13	12.91	1.78	1.6	0.45
9/9/2009	56	2.02				0.37			0.55	
11/9/2009	58	6.49							0.21	
13/9/2009	60	6.74	0.64							
15/9/2009	62	2.02				0.37			0.55	
18/9/2009	65	3.52							1.19	16.72

Table B-10 Influent VFAs composition data throughout the operation period (cont.)

D/M/Y	Time (day)	Influent								
		Ac	Pr	Isobu	Bu	Isova	Va	Isoca	Ca	Hep
25/9/2009	72	5.36	0.88		0.14				0.38	
29/9/2009	76	3.85	0.92						0.18	
1/10/2009	78	3.11	0.54						0.49	
3/10/2009	80	3.36	4.8	31.73	4.23	3.8	3.28	7.67	25.53	14.72
5/10/2009	82	2.08	0.52						0.91	1.57
9/10/2009	86	1.94	0.6						0.13	0.14
13/10/2009	90	1.36								
16/10/2009	93	1.42	0.83							
20/10/2009	97		1							
23/10/2009	100	2.51	5.2	0.31						
26/10/2009	103	2.72	0.82		0.26			0.4	1.4	2.04
28/10/2009	105	1.85							0.26	0.74
30/10/2009	107	2.54	1.63	0.27						
2/11/2009	110	1.44								
6/11/2009	114	2.3	1.29							
10/11/2009	118	2.09	0.66							
13/11/2009	121	2.51	5.32	0.31						
16/11/2009	124	2.32	0.82		0.26			0.4	1.4	2.04
19/11/2009	127	5.81	5.57							
24/11/2009	132	2.1	12.07	11.08	1.47				11.13	
27/11/2009	135	6.45	6.64	3.7	5.84	4.11	7.3	0.01	9.2	0.05
30/11/2009	138	2.38	0.79		0.79		1.3	0	2.58	6.4
1/12/2009	139	1.27	0.65					0.89	0.26	
4/12/2009	142		1.56				0.59		0.26	
8/12/2009	146		0.56		0.12				0.4	
11/12/2009	149	0.47				3.78	0.78			
15/12/2009	153						4.62			
18/12/2009	156						0.66			
22/12/2009	160		1.06				5.11			
25/12/2009	163		1.27				4.75		0.82	
28/12/2009	166						0.63			
30/12/2009	168		0.82				4.57			
1/1/2010	170		0.79				3.97			
4/1/2010	173						4.34	0.21	0.57	
11/1/2010	180						4.29	0.13	0.14	
18/1/2010	187				0.17		4.22	1.87	4.22	
25/1/2010	194		0.58			4.83		0.13		
1/2/2010	201		0.64		0.83		0.63		0.2	
8/2/2010	208						4.57			
15/2/2010	215			1.35			3.97			

124

Table B- 11 Effluent VFA compositions data throughout the operation period (cont.)

D/M/Y	Time (day)	Effluent 6h-HRT									Effluent 12h-HRT								
		Ac	Pr	Isobu	Bu	Isova	Va	Isoca	Ca	Hep	Ac	Pr	Isobu	Bu	Isova	Va	Isoca	Ca	Hep
17/7/2009	2	1.3							0.14		1.8				1.19			0.66	
21/7/2009	6	2.27							2.15		2.15							0.64	
24/7/2009	9	4.8				3.21			1.34		2.38				3.33			0.46	
28/7/2009	13	4.57									1.46								
31/7/2009	16	4.57							0.16		4.19							0.33	
4/8/2009	20	2.08			1.37				0.63		3.13			0.37					
7/8/2009	23	2.43									2.97								
10/8/2009	26	3.86							2.1		3.71							0.28	
11/8/2009	27	2.88							0.12		2.42			2.38				0.11	
14/8/2009	30	2.59									4.11								
18/8/2009	34	2.29									2.94	0.2	0.42						
21/8/2009	37	1.79									4.82								
25/8/2009	41	3.06				0.59			0.54		1.98								
28/8/2009	44	2.82				0.87			0.55		1.3				1.87	4.19	0.17	4.06	
1/9/2009	48	2.75				1.13			0.64		2.58				0.52			0.17	
4/9/2009	51	3.86		0.26	0.63				0.86	1.34	5.69		0.28	0.23				0.84	0.21
9/9/2009	56	7.27			0.3	0.83			2.12		3.26				1.11			0.69	
11/9/2009	58	4.1							0.24		8.71							0.12	
13/9/2009	60	2.7	0.86								3.33								
15/9/2009	62	4.04				1.92			0.99		1.8				1.19			0.66	
18/9/2009	65	3.6							0.25		2.52							0.1	
22/9/2009	69	2.65							0.32		2.76							0.12	
25/9/2009	72	0.91	0.9						0.34		1.31			0.11				0.36	
29/9/2009	76	2.94			0.22				0.46		2.86								0.42
1/10/2009	78	3.76	0.62		0.16				0.34		4.33	0.77		0.82		0.19	1.06	2.04	3.26
3/10/2009	80	2.86	15.52	18.05	3.96	7.04	2.92	1.74	0.39		11.66	1.38	26.1	3.62	2.76			12.16	
5/10/2009	82	1.84							0.53	0.71	0.59							0.13	0.34

125

Table B-11 Effluent VFA compositions data throughout the operation period (cont.)

D/M/Y	Time (day)	Effluent 6h-HRT									Effluent 12h-HRT								
		Ac	Pr	Isobu	Bu	Isova	Va	Isoca	Ca	Hep	Ac	Pr	Isobu	Bu	Isova	Va	Isoca	Ca	Hep
9/10/2009	86	3.23	0.87						0.15	0.28	1.35								
13/10/2009	90																		
16/10/2009	93	2.52	0.8								1.86	1.45							
20/10/2009	97	1.5	1.56									2.1							
23/10/2009	100	1.62	0.93								1.65	0.89							
26/10/2009	103	2.14	0.53						0.62	0.84	1.85							0.26	0.74
28/10/2009	105	1.65	1.48						0.26	0.43	1.93	0.88						0.42	
30/10/2009	107	1.56									1.29	0.52							
2/11/2009	110																		
6/11/2009	114	1.59								0.03	1.44								
10/11/2009	118	2.1	1.64								2.11	0.7							
13/11/2009	121	1.62	0.93								1.65	0.89							
16/11/2009	124	2.14	0.52						0.62	0.84	1.65	1.48						0.26	0.43
19/11/2009	127	3.63	0.99	0.46							2.54	1.63	0.27						
24/11/2009	132	2.57	4.7	5.06	0.88				0.14	0.72	1.42	4.57	20.26	2.31	11.09			8.96	0.75
27/11/2009	135	4.46	0.76	4.23		1.17	4.44		0.18	4.35	5.94	3.93	0.68	2.23	0.38	3.81		5.43	5.62
30/11/2009	138		0.71			0.43		0.2		1.17	2.7	0.98		0.08			0.23	0.56	
1/12/2009	139	3.42	1		0.34				0.14		3.86	1.13		0.32		0.03			
4/12/2009	142		1.56				0.59		0.26		3.85	0.95				5.63	0.2	0.1	
8/12/2009	146	1.39	0.46				2.27	0.18	0.03		2	1.67	0.11	0.18			0.01	0.03	1.58
11/12/2009	149						4.24					1.04				3.78	0.17		
15/12/2009	153		0.55				4.52	0.01				0.48				4.28			
18/12/2009	156		0.52				4.87					0.08				4.71		0.33	0.2
22/12/2009	160						4.34		0.04							1.02	1.26	4.67	
25/12/2009	163		0.46				1.73		2.01							1.63	1.5	3.11	
28/12/2009	166						2.27		0.14			0.95				1.13	0.2	0.1	
30/12/2009	168		0.55				4.24		0.26							1.67	0.01	0.03	

126

Table B-11 Effluent VFA compositions data throughout the operation period (cont.)

D/M/Y	Time (day)	Effluent 6h-HRT									Effluent 12h-HRT								
		Ac	Pr	Isobu	Bu	Isova	Va	Isoca	Ca	Hep	Ac	Pr	Isobu	Bu	Isova	Va	Isoca	Ca	Hep
1/1/2010	170		0.52				4.52		0.03							1.04	0.18	0.03	
4/1/2010	173						4.43	0.37	0.18	0.35									
11/1/2010	180		0.59			1.17						1.06				4.28		0.03	
18/1/2010	187		1.81	0.47			4.29		0.38			0.59				4.48			
25/1/2010	194						5.19	0.56	3.4							4.13		0.53	
1/2/2010	201						3.75	0.47	0.1			0.59				2.4			
8/2/2010	208				0.27		4.24									4.74	0.14		
15/2/2010	215		1.33	3	0.14		1.26	0.05	0.5			0.89	3.14			3.5	0.63	0.21	

Note: acetic (Ac), propionic (Pr), iso-butysic (isobu), butyric(bu), iso-valeric (iso-va), valeric, iso-caproic (iso-cap), caproic (cap) and haptanioic (hep) acids.

BIOGRAPHY

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