BIOTRANSFORMATION OF CAFFEINE, GLICLAZIDE AND PRAZOSIN IN AN UP-FLOW ANAEROBIC SLUDGE BLANKET REACTOR

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DEDICATION

This thesis is dedicated to my mother and late father who always encouraged me to pursue my dreams and keep me in their prayers. I also dedicate this thesis to my husband and children who has been patient and constantly supported me to sail through these postgraduate years.

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ABSTRACT

Up-flow anaerobic sludge blanket (UASB) reactors are largely utilised as an anaerobic reactor configuration to efficiently treat various wastewater streams. However, previous UASB operations for the removal of pharmaceutical compounds at low hydraulic retention time (HRT) resulted in poor chemical oxygen demand (COD) and removal efficiencies for the investigated compounds. Anaerobic process performance has also been found to be vulnerable towards pharmaceutical compounds at high concentration levels but there is no evidence to indicate if the same effects will be induced when other compounds are present at trace concentration levels. This research aims to establish the relationship between pharmaceutical compound removal, influencing parameters, and anaerobic process performance. In this research, caffeine, gliclazide, and prazosin were selected based on their environmental occurrences, persistency, and toxicity. The compounds were investigated as a mixture to simulate their concurrent occurrences in the same actual wastewater stream. The first part of the research investigated the biodegradability of the three compounds in an anaerobic batch experiment. Synthetic wastewater and inoculum mixture were spiked with 1 mg/L of mixed pharmaceutical compounds and incubated at mesophilic condition (37°C) for 90 days. All compounds achieved removal through biotransformation between 44 - 99% by the end of the incubation period. The second part of the research was commenced by running five experimental phases in an acclimatised laboratory-scale UASB reactor. Phases I - V were carried out to assess the effect of pharmaceutical concentrations (0.1 – 1 mg/L), HRT (36 – 48 hours) and different reducing conditions (predominant methanogenic and simultaneously reducing conditions) to the removal of pharmaceutical compounds and UASB process performance. Overall, biotransformation remained the predominant removal pathway in the laboratory-scale experiments despite the changes in pharmaceutical concentrations and HRT. Only gliclazide recorded an improvement of biotransformation up to 99% under simultaneous reducing conditions compared to that in predominant methanogenic conditions. The UASB reactor was also operating in a stable condition (COD removal efficiency of 93 ± 2%) but fluctuations were recorded in the production of individual volatile fatty acids (VFAs) and biogas. Microbial assessment through 16s rRNA sequencing justified the changes in VFAs production based on the effect of the pharmaceutical compounds towards the growth of hydrolytic and fermentative bacteria. The compounds also shifted the composition of methanogenic archaea by favouring hydrogenotrophic methanogens (59 - 72%) over acetoclastic methanogens (15 - 31%) under predominant methanogenic conditions.

ABSTRAK

Reaktor enapcemar anaerobik aliran atas (UASB) digunakan sebagai konfigurasi reaktor anaerobik secara meluas untuk merawat pelbagai aliran air sisa dengan cekap. Walau bagaimanapun, operasi UASB untuk penyingkiran sebatian farmaseutikal sebelum ini pada masa pengekalan hidraulik (HRT) yang rendah menyebabkan penyingkiran permintaan oksigen kimia (COD) dan sebatian farmaseutikal yang rendah. Prestasi proses anaerobik juga didapati terjejas disebabkan oleh kehadiran sebatian farmaseutikal pada tahap kepekatan tinggi tetapi tiada bukti jika kesan yang sama akan terhasil sekiranya sebatian lain hadir pada tahap kepekatan yang rendah. Matlamat penyelidikan ini adalah untuk mengesahkan hubungan antara penyingkiran sebatian farmaseutikal, pengaruh parameter dan prestasi proses anaerobik. Kafein, glikazida dan prazosin dipilih untuk penyelidikan ini berdasarkan pengesanan di alam sekitar secara berterusan dan ketoksikannya. Sebatian-sebatian ini telah disiasat sebagai campuran untuk mensimulasikan kejadian mereka dalam aliran air sisa sebenar. Bahagian pertama penyelidikan ini telah menyiasat kebolehbiodegradan ketiga-tiga sebatian dalam eksperimen kelompok anaerobik. Air sisa sintetik dan campuran inokulum telah dicampurkan dengan 1 mg/L campuran sebatian farmaseutikal dan diinkubasi pada keadaan mesofilik (37°C) selama 90 hari. Semua sebatian mencapai penyingkiran melalui biotransformasi antara 44 - 99% pada akhir tempoh inkubasi. Bahagian kedua penyelidikan dijalankan melalui lima fasa eksperimen dalam reaktor UASB skala makmal yang telah diaklimatasi. Fasa I - V telah dilaksanakan untuk menilai kesan kepekatan farmaseutikal (0.1 – 1 mg/L), HRT (36 – 48 jam) dan keadaan reduktasi yang berbeza (keadaan metanogenik dan keadaan reduktasi bercampur) kepada penyingkiran sebatian farmaseutikal dan prestasi proses UASB. Secara keseluruhannya, biotransformasi kekal sebagai laluan penyingkiran utama dalam eksperimen berskala makmal walaupun kepekatan farmaseutikal dan HRT telah diubah. Hanya glikazida mencatatkan peningkatan biotransformasi sehingga 99% di bawah keadaan reduktasi bercampur berbanding dengan keadaan metanogenik yang dominan. Reaktor UASB juga beroperasi dalam keadaan yang stabil (penyingkiran COD pada takat 93 ± 2%) tetapi turun naik telah direkodkan bagi penghasilan asid lemak meruap (VFA) individu dan biogas. Penilaian mikrob melalui *16s rRNA sequencing* membuktikan perubahan pengeluaran VFA berdasarkan kesan sebatian farmaseutikal terhadap pertumbuhan bakteria hidrolitik dan fermentatif. Sebatian-sebatian itu juga mengalihkan komposisi arkaea metanogenik dengan pertumbuhan metanogen hidrogenotropik (59 - 72%) yang lebih tinggi berbanding metanogen asetoklastik (15 - 31%) di bawah keadaan metanogenik yang dominan.

TABLE OF CONTENTS

TITLE		PAGE
DECLARATION		**iii**
DEDICATION		**iv**
ACKNOWLEDGEMENT		**v**
ABSTRACT		**vi**
ABSTRAK		**vii**
TABLE OF CONTENTS		**viii**
LIST OF TABLES		**xiii**
LIST OF FIGURES		**xv**
LIST OF ABBREVIATIONS		**xviii**
LIST OF SYMBOLS		**xx**
LIST OF APPENDICES		**xxii**
CHAPTER 1	**INTRODUCTION**	**1**
1.1	Problem Background	1
1.2	Problem Statement	4
1.3	Research Questions	5
1.4	Research Objectives	6
1.5	Scope of Research	7
1.6	Significance of the Research	9
CHAPTER 2	**LITERATURE REVIEW**	**10**
2.1	Introduction	10
2.2	Pharmaceutical Compounds in the Water Environment	10
	2.2.1 Occurrences of Pharmaceutical Compounds in the Environment	10
	2.2.2 Current Treated Effluent Standards in Malaysia	13
	2.2.3 Pharmacology of Caffeine, Gliclazide and Prazosin	14

2.2.3.1 Caffeine 14

2.2.3.2 Gliclazide 15

2.2.3.3 Prazosin 16

2.2.4 Impact of Caffeine, Gliclazide and Prazosin to the Environment 16

2.3 Anaerobic Digestion 19

2.3.1 Up-flow Anaerobic Sludge Blanket (UASB) 23

2.4 Fate of Pharmaceutical Compounds in Anaerobic Digestion 25

2.4.1 Biodegradation and Biotransformation of Pharmaceutical Compounds 26

2.4.2 Sorption of Pharmaceutical Compounds 30

2.4.3 Residuals in Wastewater Effluent from Anaerobic Process 33

2.5 Factors Affecting the Removal of Pharmaceutical Compounds 34

2.5.1 Chemical Structure of Pharmaceutical Compound 37

2.5.2 Reducing Conditions in Anaerobic Treatment 38

2.5.3 Acclimatisation 41

2.5.4 Operational pH 43

2.5.5 Sludge Characteristics 44

2.5.6 Hydraulic Retention Time (HRT) 45

2.5.7 Temperature 47

2.6 Effect of Pharmaceutical Compounds to Anaerobic Process 47

2.6.1 Methane Gas Production 48

2.6.2 Utilisation of Substrates for Methane Conversion 52

2.6.3 Shift in Microbial Diversity 53

2.7 Research Involving Removal of Caffeine, Gliclazide and Prazosin 56

2.8 Summary of Literature Review 60

CHAPTER 3 RESEARCH METHODOLOGY 63

3.1 Introduction 63

3.2 Experimental Design 63
3.3 Materials 65
3.3.1 Chemicals 65
3.3.1.1 Synthetic wastewater 65
3.3.1.2 Analytical chemicals 66
3.3.1.3 Pharmaceutical compounds 67
3.3.2 Inoculum 69
3.4 Batch Experiment 71
3.5 UASB Bioreactor Start-up 72
3.6 Laboratory-scale Experiments 76
3.7 UASB Monitoring and Sampling Procedure 79
3.8 Analytical Method 80
3.8.1 Pharmaceutical Compounds Analysis 80
3.8.1.1 Liquid Chromatography Analysis 81
3.8.1.2 Preparation for Aqueous Samples 83
3.8.1.3 Preparation for Solid Samples 85
3.8.1.4 Method validation 85
3.8.2 Anaerobic Performance Monitoring 87
3.8.3 Microbial Analysis Using 16 rRNA Sequencing 90
3.9 Mathematical Computations 91

CHAPTER 4 BIOTRANSFORMATION OF CAFFEINE, GLICLAZIDE AND PRAZOSIN IN BATCH AND UASB EXPERIMENTS 94

4.1 Introduction 94
4.2 Initial Characteristics of Synthetic Wastewater and Inoculum 94
4.3 Biodegradability of Caffeine, Gliclazide and Prazosin in the Anaerobic Mesophilic Batch Experiment 95
4.3.1 Removal of the Pharmaceutical Compounds in the Batch Experiment 96
4.3.2 Kinetic Model of the Pharmaceutical Compounds in the Batch Experiment 100
4.3.3 Anaerobic Performance in the Batch Experiment 102

4.3.4 Summary of Batch Experiment Results 107

4.4 UASB Bioreactor Start-up 107

4.5 Biotransformation of Caffeine, Gliclazide and Prazosin in Laboratory-Scale UASB bioreactor 112

4.5.1 Removal of Caffeine, Gliclazide and Prazosin in Phases I – V Experiments 112

4.5.2 Comparison of Removal Efficiencies for Caffeine, Gliclazide and Prazosin in this Research and Literature 116

4.5.3 Kinetics of pharmaceutical compounds in UASB reactor 118

4.5.4 Factors Affecting the Removal of the Compounds in Phases I – V Experiments 120

4.5.4.1 Chemical Structure of the Pharmaceutical Compounds 120

4.5.4.2 Effect of HRT to the Pharmaceutical Compounds 122

4.5.4.3 Role of Reducing Conditions for the Removal of Pharmaceutical Compounds 123

4.5.4.4 Methanogenic Activity and Biotransformation of Pharmaceutical Compounds 124

4.6 Effect of Caffeine, Gliclazide and Prazosin Towards UASB Process Performance 125

4.7 Summary of the Results from Batch and Laboratory-Scale UASB Reactor Experiments 132

CHAPTER 5 THE INFLUENCE OF PHARMACEUTICAL COMPOUNDS TO THE MICROBIAL DIVERSITY IN UASB REACTOR 133

5.1 Introduction 133

5.2 Development of Granular Sludge 133

5.3 Results from 16s rRNA Sequencing 134

5.3.1.1 Quality of Extracted gDNA 135

5.3.1.2 Alpha-Diversity and Beta-Diversity 136

5.3.1.3 Taxonomical Unit Information 138

5.4 Pharmaceutical Compound Removal and Microbial Communities 143

5.5 Summary of 16s rRNA Sequencing Results 145

CHAPTER 6 CONCLUSION AND RECOMMENDATIONS 147

6.1 Research Outcomes 147

6.2 Contributions to Knowledge 148

6.3 Future Works 149

6.4 Limitations of This Research 149

REFERENCES 151

LIST OF PUBLICATIONS 205

LIST OF TABLES

TABLE NO.	TITLE	PAGE
Table 2.1	Detections of caffeine, gliclazide and prazosin within the same source in Malaysian water environment	11
Table 2.2	Removal efficiencies of caffeine, gliclazide and prazosin in existing conventional WWTPs (Al-Qaim et al., 2015)	13
Table 2.3	Pharmacology of caffeine, gliclazide and prazosin	15
Table 2.4	Impact of occurring trace pharmaceutical compounds in the water bodies to aquatic species	18
Table 2.5	Advantages and disadvantages of different anaerobic technologies	22
Table 2.6	UASB bioreactor performance for various type of wastewater	25
Table 2.7	Removal performance of pharmaceutical compounds in UASB bioreactor	35
Table 2.8	The effect of pH from 5 - 8 to the degree of sorption in anaerobic sludge digestion as reported by Narumiya et al. (2013)	44
Table 2.9	The effect of pharmaceutical compounds to methane production and VFAs accumulation in mesophilic incubation experiments	49
Table 2.10	Removal efficiencies of caffeine, gliclazide and prazosin in previous laboratory wastewater treatment studies	57
Table 3.1	Formulation of synthetic wastewater modified from Kodera et al. (2017)	66
Table 3.2	Physico-chemical characteristics of caffeine, gliclazide and prazosin (Wishart et al., 2018)	67
Table 3.3	Derivation of individual standards stock solution (U.S. EPA, 2007a)	68
Table 3.4	Derivation of mixed standards solution for 1000 mg/L and 1 mg/L	68
Table 3.5	Calculation for preparation of stock solution of pharmaceutical compounds	69
Table 3.6	Dilution of synthetic wastewater for the batch experiment	71

Table 3.7	Dilution of synthetic wastewater for the UASB reactor operation	76
Table 3.8	Operational conditions for the laboratory-scale experiments	77
Table 3.9	Dilution calculation for pharmaceutical compounds between Phases I – V	78
Table 3.10	Flowrate calculation corresponding to HRT	79
Table 3.11	Calculation of KNO_3 for Phase V	79
Table 3.12	Summary of LC analytical method	82
Table 3.13	Method validation results for LC analysis	87
Table 3.14	Methods and instruments related to the chemical analysis of anaerobic parameters	88
Table 4.1	Characteristics of synthetic wastewater	95
Table 4.2	Characteristics of anaerobic digested sludge	95
Table 4.3	Concentration of pharmaceutical compounds in effluent and biomass after 90 days	100
Table 4.4	Statistical analysis of each pharmaceutical compound fitted to pseudo-first order and second order kinetic model (α = 0.05)	101
Table 4.5	Distribution of pharmaceutical compounds in solids at end of each phase	115
Table 4.8	Removal of caffeine, gliclazide and prazosin in this research and literature	117
Table 4.6	Pseudo-first order and second order biotransformation rate constants for the pharmaceutical compounds in Phases I - V	119
Table 4.7	Relationship between methanogenic activity and biotransformation rate of pharmaceutical compounds in the UASB reactor	125
Table 5.1	Details of the gDNA products	135
Table 5.2	Total sequencing tags and OTUs identified in each sample	135
Table 5.3	Alpha-diversity values for respective conditions	137

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
Figure 2.1	Schematic diagram of route of pharmaceutical compounds into the water environment via wastewater treatment process	12
Figure 2.2	Chemical structure of caffeine, gliclazide and prazosin	14
Figure 2.3	Anaerobic digestion process diagram adapted from Lohani and Havukainen (2017)	20
Figure 2.4	Conceptual diagram of UASB bioreactor (Dutta et al., 2018)	24
Figure 2.5	Fate of pharmaceutical compounds in anaerobic digestion	26
Figure 2.6	Chemical reaction in the acetyl-CoA pathway (Martin, 2020)	28
Figure 2.7	Plots of concentration of sulfamethoxazole (SMX), erythromycin (ERY), bisphenol A (BPA) and fluoxetine (FLX) (Gonzalez-Gil et al., 2018b)	29
Figure 2.8	Molecular structure of micropollutants containing different functional groups	38
Figure 2.9	Biotransformation performance of caffeine (CAF), ibuprofen (IBP), carbamazepine (CBZ), 4-nonylphenol (NP) and sulfamethoxazole (SMX) in methanogenic (MTG), sulfate-reducing (SO_4), nitrate-reducing (NO_3) and manganese-reducing (Mn(IV)) conditions	40
Figure 2.10	Removal performance of diclofenac and ibuprofen in MAD/TAD (Samaras et al., 2014), AnMBR (Monsalvo et al., 2014), Pilot AD (Phan et al., 2018), and RT AD (Yang et al., 2017a) with VS/TS range 0.64 – 0.81	46
Figure 2.11	Diversion of aceticlastic pathway to hydrogenotrophic pathway through oxidation of VFAs to carbon dioxide and hydrogen in the presence of pharmaceutical compounds	56
Figure 3.1	Experimental design for this research	64
Figure 3.2	Location from Google Earth (a) and picture of full-scale anaerobic CSTR tanks (b) at IWK Pantai 1 Sewage Treatment Works, Kuala Lumpur	70

Figure 3.3	Set-up of anaerobic medium and inoculum mixture spiked with mixture of pharmaceutical compounds for the batch experiment	73
Figure 3.4	Actual UASB reactor used for the laboratory-scale experiment	74
Figure 3.5	Conceptual diagram of the laboratory-scale UASB reactor	75
Figure 3.6	Waters ACQUITY UPLC-QDa liquid chromatography instrument	82
Figure 3.7	SPE setup for sample pre-concentration	84
Figure 3.8	Thermo Scientific ICS 5000+ ion chromatography instrument	89
Figure 3.9	Perkin Elmer Clarus® 690 GC-TCD instrument	90
Figure 4.1	Removal efficiencies of pharmaceutical compounds throughout incubation period under anaerobic condition	97
Figure 4.2	Distribution of biotransformed and sorbed fraction of caffeine, gliclazide and prazosin throughout incubation period under anaerobic condition	98
Figure 4.3	COD removal efficiency in the batch experiment	103
Figure 4.4	Production of VFAs in the batch experiment	104
Figure 4.4	Biogas compositions in the batch experiment	106
Figure 4.7	COD removal throughout the UASB start-up	108
Figure 4.8	pH and VSS/TSS ratio throughout the UASB start-up	109
Figure 4.9	Distribution of individual VFA throughout the UASB start-up	110
Figure 4.10	Daily biogas production throughout the UASB start-up	111
Figure 4.11	Total removal, degree of biotransformation and degree of sorption for respective compounds at the end of UASB Phases I – V	113
Figure 4.12	Residual concentration of caffeine in treated effluent during UASB Phases I – V	114
Figure 4.13	Residual concentration of gliclazide in treated effluent during UASB Phases I – V	114
Figure 4.14	Residual concentration of prazosin in treated effluent during UASB Phases I – V	115
Figure 4.15	COD removal performance during UASB Phases I - V	127

Figure 4.16	pH inoculum, pH effluent and VSS/TSS ratio during UASB Phases I - V	128
Figure 4.17	Distribution of individual VFA during UASB Phases I – V	128
Figure 4.18	Total nitrogen and ammoniacal nitrogen concentration in treated effluent during UASB Phases I – V	130
Figure 4.19	Biogas production during UASB Phase I – V	131
Figure 5.1	Image and average diameter of matured granules from UASB reactor in respective experimental conditions	134
Figure 5.2	Beta-diversity PCoA graph for Baseline (BUASB), Phase II (1UASB), Phase IV (2UASB) and Phase V (3UASB)	137
Figure 5.3	Distribution of archaeal genera in the UASB reactor	139
Figure 5.4	Distribution of bacteria phylum in all samples	141
Figure 5.5	Distribution of bacterial genera in all samples	142

LIST OF ABBREVIATIONS

AnMBR	-	Anaerobic membrane bioreactor
ANOVA	-	Analysis of variance
AnSBR	-	Anaerobic sequencing batch reactor
ATC	-	Anatomical therapeutic chemical
BOD	-	Biochemical oxygen demand
CAS	-	Chemical abstract service
CAS	-	Conventional activated sludge
CCR	-	Carbon catabolite repression
CSTR	-	Continuously stirred tank reactor
Cd (II)	-	Cadmium (II)
COD	-	Chemical oxygen demand
CO_2	-	Carbon dioxide
DDD	-	Defined daily dose
DO	-	Dissolved oxygen
EDG	-	Electron donating groups
EPS	-	Extracellular polymeric substances
EWG	-	Electron withdrawing groups
Fe	-	Iron
GC-TCD	-	Gas chromatography-thermal conductivity detector
HLB	-	Hydrophilic-lipophilic balance
HRT	-	Hydraulic retention time
ICP-OES	-	Inductively coupled plasma – optical emission spectrometry
IQL	-	Instrumentation quantification limit
LC	-	Liquid chromatography
LD_{50}	-	Lethal dose 50
LOQ	-	Limit of quantification
MAD	-	Mesophilic anaerobic digester
MDL	-	Method detection limit
Mn	-	Manganese
n.d.	-	Not detected

NGS	-	Next generation sequencing
NO_3	-	Nitrate
NPE	-	Nonylphenol ethoxylates
NSAID	-	Non-steroidal anti-inflammatory drug
OLR	-	Organic loading rate
OTU	-	Operational taxonomic units
PCR	-	Polymerase chain reaction
PTSD	-	Post-traumatic stress disorder
RSD	-	Relative standard deviation
RT	-	Retention time
RT AD	-	Recuperative thickening anaerobic digesters
SBR	-	Sequencing batch reactor
Se	-	Selenium
SO_4	-	Sulfate
SPE	-	Solid phase extraction
SRT	-	Sludge retention time
SSRI	-	Selective serotonin reuptake inhibitor
TAD	-	Thermophilic anaerobic digesters
TN	-	Total nitrogen
TS	-	Total solids
TSS	-	Total suspended solids
UASB	-	Up-flow anaerobic sludge blanket
U.S.EPA	-	United States Environmental Protection Agency
VFA	-	Volatile fatty acid
VS	-	Volatile solids
VSS	-	Volatile suspended solids
WWTP	-	Wastewater treatment process
Zn(II)	-	Zinc (II)

LIST OF SYMBOLS

α	-	Confidence level
C	-	Concentration of pharmaceutical compounds
C_A	-	Concentration of pharmaceutical compounds in aqueous phase
C_{abio}	-	Fraction of removed pharmaceutical compounds through abiotic reaction
C_{bio}	-	Fraction of biotransformed pharmaceutical compounds
C_o	-	Initial concentration of pharmaceutical compounds
C_{res}	-	Residual pharmaceutical compounds in treated effluent
C_s	-	Concentration of pharmaceutical compound in solid phase
CF	-	Concentration factor
d	-	day
h	-	hour
kg	-	Kilogram
kg COD/m^3.d	-	Kilogram chemical oxygen demand per cubic meter per day
k_{biol}	-	Biotransformation rate constant
K_d	-	Distribution coefficient
K_{ow}	-	Octanol-water partitioning coefficient
L CH_4/d	-	Liter methane per day
L/g VSS.d	-	Liter per gram volatile suspended solids per day
µg/L	-	Microgram per liter
µL/mg	-	Microliter per microgram
mg	-	Milligram
mg/kg	-	Milligram per kilogram
mg/L	-	Milligram per liter
M_w	-	Molecular weight
ng/L	-	Nanogram per liter
pK_a	-	Dissociation constant
R^2	-	Coefficient of determination
RE	-	Recovery

X_{VSS}	-	Concentration of volatile suspended solids

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
Appendix A	Method Development for Liquid Chromatography Analysis	174
Appendix B	Method Determination for Solid Phase Extraction (SPE)	179
Appendix C	Calibration Curves for Liquid Chromatography Analysis	183
Appendix D	Characteristics of Synthetic Wastewater and Inoculum	185
Appendix E	Batch Experiment Monitoring Data	186
Appendix F	Monitoring Data for the UASB Bioreactor Start-up	189
Appendix G	Pharmaceutical Compounds Data for UASB Experimental Phases I - V	192
Appendix H	Biotransformation Rate Constants of Pharmaceutical Compounds in UASB Reactor	194
Appendix I	Methanogenic Activity and Biotransformation Rate of Pharmaceutical Compounds	195
Appendix J	Monitoring Data for UASB Experimental Phases I - V	196
Appendix K	Gel Electrophoresis of PCR Products from gDNA Extraction	199
Appendix L	Theory of Alpha-diversity and Beta-diversity	200
Appendix M	Distribution of Microbial Communities	202

CHAPTER 1

INTRODUCTION

1.1 Problem Background

Pharmaceutical compounds are currently among the compounds of emerging concerns due to their detections in the global water environment. Malaysia is not excluded from these occurrences as similar detections have been recorded at concentrations between nanogram per liter (ng/L) to micrograms per liter (μg/L) (Praveena et al., 2020). Numerous compounds were found within the same water and wastewater samples which included psychostimulant caffeine, anti-diabetic drug gliclazide and anti-hypertensive drug prazosin. From the literature, the detections of caffeine were recorded at maximum concentration of 20.6 μg/L, gliclazide up to 99.2 ng/L, and prazosin up to 117 ng/L within the same wastewater effluent and river tributaries (Hashim et al., 2021). The occurrences of the three compounds correlated with their high consumption in Malaysia (Al-Qaim et al., 2018d; Ministry of Health Malaysia, 2020). These compounds may enter the wastewater stream through direct or indirect discharge at point source, namely sewage, sullage and leachate.

Previous occurrence studies discovered that existing conventional wastewater treatment processes (WWTPs) have varying removal efficiencies for caffeine, gliclazide and prazosin, from poor to more than 90% removal (Al-Qaim et al., 2015; Tahrim et al., 2018). These conventional WWTPs incorporated two-stage treatment whereby physical process is applied for primary treatment and combination of chemical and biological processes for secondary treatment (Grady Jr. et al., 2011). Common conventional practices also operate activated sludge for aerobic process in the biological treatment (Grady Jr. et al., 2011). These WWTPs were initially built to achieve the acceptable limit of discharge for the parameters specified for treated effluent which do not include the limit for pharmaceutical compounds. The original

design did not include the need to treat pharmaceutical compounds and could not achieve an effective removal for the compounds present in the wastewater stream.

Concerns arose as trace pharmaceutical compounds are continuously discharged to the environment to the extent that they can potentially harm directly impacted species especially aquatic species when ingested over a long period of exposure (U.S. EPA, 2022). Some of the observed impacts by these three compounds included stunted fish hatchling growth (Lee and Wang, 2015), hormonal disruptions (Kubacka et al., 2021) and behavioural changes (Mei et al., 2021), despite being at much lower concentration than their lethal toxicity concentration level. Furthermore, the compounds were persistent in the water cycle as they were present in drinking water (Praveena et al., 2020) and transferable to agricultural soils via recycled water and biosolids (Mrozik and Stefanska, 2014).

The treatment of caffeine, gliclazide and prazosin can result into formation of multiple by-products. Caffeine can be metabolised into other methylxanthine derivatives like paraxanthine, theobromine and theophylline (Wishart et al., 2018). Biological treatment was found to hydrolyse gliclazide to form hydroxyl and carboxyl by-products (Mrozik and Stefanska, 2014), while prazosin can transform to piperazine and quinazoline by-products (Mohd Mohsi et al., 2019). Their ability to transform emphasised that biotransformation is a possible removal pathway for these compounds if complete biodegradation is not achieved.

Researchers studied on different biological treatment methods to improve the removal of caffeine, gliclazide and prazosin. Only caffeine was actively studied in anaerobic treatment out of these three compounds. Anaerobic process achieved nearly complete removal for caffeine in a mesophilic (35°C) batch experiment (He et al., 2018), and good removal performance in mesophilic (Phan et al., 2018) and thermophilic (55°C) (Chen et al., 2018a) anaerobic reactors. Gliclazide has low removal efficiencies of below 40% in constructed wetlands (Petrie et al., 2018) and aerobic sequencing batch reactor (SBR) (Mat Zaini, 2020). Prazosin was also poorly removed in aerobic SBR (Mat Zaini, 2020). Comparatively, anaerobic digestion has a

good record in removing recalcitrant compounds through biotransformation (Harb et al., 2019) and served as an attractive approach to treat these three compounds.

Anaerobic digestion operates in the absence of oxygen and consists of four intermediate stages which are hydrolysis, fermentation (acidogenesis and acetogenesis) and methanogenesis. Anaerobic process showed capability in treating various wastewater streams such as sewage sludge (Hanum et al., 2019), pharmaceutical wastewater (Afridi et al., 2018), palm oil mill effluent (Halim and Yong, 2018), and wastewater from livestock and farming (Marzuki et al., 2021). The basic operation of anaerobic process consumes minimal energy and has biogas production potential for energy recovery (Grady Jr. et al., 2011). Up-flow anaerobic sludge blanket (UASB) is among the frequently applied anaerobic process configuration. According to the literature, UASB displayed capability in accomplishing more than 90% of chemical oxygen demand (COD) removal efficiencies for different types of wastewaters (Ma et al., 2018; Wu et al., 2018; Jiraprasertwong et al., 2019).

Biotransformation of pharmaceutical compounds in anaerobic digestion may unlikely be achieved through direct metabolism. This is because the compounds are often detected at low concentration which is insufficient to be the main substrate for redox reaction. Instead, co-metabolism may drive the biotransformation process. Metabolic reactions between microorganisms and the main substrates in the wastewater produce enzymes which may be utilised for biotransformation of pharmaceutical compounds (Tran et al., 2018). These substrates contain electron acceptors which induce reducing conditions in anaerobic reactors. The nature of each compound having different functional groups in its chemical structure may favour specific electron acceptors for the biotransformation process (Gonzalez-Gil et al., 2018a). Thus, this emphasised that physico-chemical characteristics of a compound and reducing conditions are the prominent factors influencing the removal of the compounds. Other factors that can affect the removal of pharmaceutical compounds in anaerobic digestion are process acclimatisation (Butkovskyi et al., 2015), operational pH (Li et al., 2021), sludge characteristics (Carballa et al., 2007), adsorption (Zhu et

al., 2018), hydraulic retention time (HRT) (Boonnorat et al., 2019), and temperature (Taboada-Santos et al., 2019).

The potential of anaerobic digestion in treating pharmaceutical compounds may come at a cost. Anaerobic experiments at mesophilic condition found that pharmaceutical compounds can disturb the process stability mainly by inhibiting methane production (Mai et al., 2018) and cause over-fermentation due to accumulation of volatile fatty acids (VFAs) (Cetecioglu and Orhon, 2018). These effects were the outcome from the changes of microbial communities triggered by the presence of pharmaceutical compounds.

1.2 Problem Statement

Removal pathway of caffeine in anaerobic digestion was well-defined whereby the compound achieved its removal mainly through biotransformation (Phan et al., 2018; Song et al., 2018b). There is limited information on the removal behaviour of gliclazide and prazosin in anaerobic digestion as they were not examined in previous anaerobic research. Other biological treatment studies involving these two compounds only defined their occurrences (Al-Qaim et al., 2018a), removal efficiencies (Mat Zaini, 2020) and transformation by-products (Mohd Mohsi et al., 2019). These studies did not consider the possibility of removal pathway via sorption as no sorption assessment was conducted for gliclazide and prazosin. It is essential to define the removal pathways of the compounds to understand and relate their removal with the influencing factors in anaerobic process.

In the application of UASB reactors at ambient temperature and HRT up to 12 h and organic loading rate (OLR) between 1.1 – 2.4 kg COD/m^3.d, the investigated compounds were removed only between 10 – 53% (Reyes-Contreras et al., 2011; Brandt et al., 2013) and achieved low COD removal efficiency (Reyes-Contreras et al., 2011). In comparison, UASB operation by Butkovskyi et al. (2015) at mesophilic condition and HRT of 35 d provided up to 99.9% removal of pharmaceutical compounds. These outcomes revealed operating temperature and HRT are important

operational parameters in treating pharmaceutical compounds, in addition to the chemical structure of the compounds and reducing conditions. The poor COD and pharmaceutical removal performance may also suggest the dependency of pharmaceutical removal performance with overall treatment performance in anaerobic reactors. The connection between these two variables were not clearly defined as research usually focus on only the removal efficiencies attained for the investigated compounds.

The relationship between pharmaceutical removal and anaerobic performance is also complex based on the effect of the compounds towards the overall treatment stability. Nevertheless, previous mesophilic studies were biased towards antibiotics (Cetecioglu and Orhon, 2018; Huang et al., 2019) which are known to exhibit antimicrobial characteristics (Tran et al., 2013). The effects were observed when examined beyond trace level concentration at concentration range of 0.5 – 1000 mg/L (Cetecioglu and Orhon, 2018; Mai et al., 2018) which do not reflect the general occurrences of other pharmaceutical compounds. These studies were also conducted in batch experiments whereby substrate limitation may not demonstrate a realistic outcome as continuous operating anaerobic reactors.

Based on these issues, the removal of pharmaceutical compounds is more than just assessing the removal efficiencies. The treatment of pharmaceutical compounds in anaerobic digestion is not a one-way solution. The interconnection between the removal of contaminants, microorganisms and treatment environment may be unique according to the investigated compounds, the presence of diverse microorganisms and the type of process implemented for the removal of the compounds.

1.3 Research Questions

The problem statements led to the following research questions:

(a) What is the relationship between the removal of selected pharmaceutical compounds (caffeine, gliclazide and prazosin) and anaerobic process performance?

(b) Do the selected pharmaceutical compounds been studied in anaerobic treatment, and how do the individual compounds behave under anaerobic condition?

(c) How does the removal of pharmaceutical compounds perform under different operational conditions?

(d) What is the relationship between anaerobic performance and the removal of pharmaceutical compounds?

(e) Do the pharmaceutical compounds affect the diversity of microorganisms in anaerobic treatment?

1.4 Research Objectives

The research questions derived the aim of this research which was to establish the relationship between the removal of selected pharmaceutical compounds and anaerobic process performance. To achieve this aim, the objectives of this research are listed as the following.

(a) To determine the biodegradability of caffeine, gliclazide and prazosin through anaerobic incubation under mesophilic condition in batch experiment.

(b) To examine biotransformation performance of the selected compounds in a laboratory-scale UASB reactor by altering the concentration of pharmaceutical compounds, HRT, and biological condition through nitrate addition.

(c) To investigate the effect of pharmaceutical compounds, HRT and nitrate addition towards anaerobic performance parameters including COD removal efficiency, VFA production, and biogas generation in the UASB reactor.

(d) To assess the influence of pharmaceutical compounds, HRT, and biological condition towards microbial diversity during the UASB operation through 16s rRNA sequencing.

1.5 Scope of Research

To achieve the listed objectives, the scope of this research was limited as described. Caffeine, gliclazide and prazosin were the pharmaceutical compounds selected for this research based on their environmental occurrences in Malaysia, their adverse effects, and different physico-chemical characteristics. Caffeine, gliclazide and prazosin were prepared and added in the same wastewater mixture to represent their simultaneous presence as found in previous studies. The compounds were assessed as a mixture following the common procedure for anaerobic treatment of pharmaceutical compounds such as stated by (Gonzalez-Gil et al., 2018b) and (Phan et al., 2018).

The removal of the compounds in anaerobic digestion was studied in batch and laboratory-scale experiments. The experiments utilised anaerobic sludge as inoculum and synthetic wastewater as feed to simulate the removal behaviour. All experiments were conducted under mesophilic condition (35 - 37°C) based on the original operating temperature of anaerobic digester sampled for anaerobic digested sludge and the agreeable performance of mesophilic temperature as reported in the literature.

For objective 1, biodegradability of caffeine, gliclazide and prazosin under anaerobic condition was examined through a 90 d incubation in a batch experiment. The incubation period was determined from the duration of 10 weeks in a batch experiment by He et al. (2018) and extended further to verify the persistency or retransformation behaviour of the compounds.

Objectives 2 – 4 were achieved by assessing the removals in a laboratory-scale UASB reactor. Initially, the UASB reactor was start-up until reaching acclimatisation before commencement of the experiments. To accomplish objective 2 and 3,

pharmaceutical compounds was introduced in the UASB reactor and varied in the concentration range of 100 – 1000 µg/L. This range was determined based on the limitation of analytical instrument while not exceeding the microgram level to avoid major disruption to the reactor performance. The concentration range also corresponded with the concentration applied by Gonzalez-Gil et al. (2018b) at 100 µg/L and up to 1000 µg/L as implemented by Cetecioglu and Orhon (2018) and Fáberová et al. (2019) to observe possible effect to the anaerobic process.

The UASB reactor operated within HRT of 36 – 48 h based on the stable performance of the reactor in treating sewage sludge prior to this research and maintain OLR up to 1.0 kg COD/m^3.d. The role of reducing conditions were also compared by conducting the experiments under predominant methanogenic condition and simultaneously reducing conditions. Simultaneously reducing conditions (nitrate and sulfate reducing conditions) were established by supplementing potassium nitrate KNO_3 as nitrate source in the wastewater. Sulfate was present in the whole experiment.

Pharmaceutical compounds in the UASB reactor were monitored periodically in both treated effluent and sludge. The results from the analysis were further investigated for the removal pathways and kinetics under each experimental condition. At the same time, the UASB reactor performance was monitored for physical, chemical, and biological parameters which included COD concentration, VFAs, other nutrients, biogas production rate and compositions. The results were analysed and consolidated to acquire the relationship between pharmaceutical compounds removal and anaerobic process performance, as well as the factors which drive the removal of the selected compounds in this research.

Lastly, objective 4 was achieved by assessing microbial composition in the sludge at each experimental condition through 16s rRNA sequencing. Microbial assessment was carried out to verify the anaerobic reducing conditions in the UASB reactor as well as identify the changes in diversity caused by the reducing conditions throughout the UASB operation.

1.6 Significance of the Research

The significance of this research is listed as stated below.

(a) The potential of anaerobic process in treating gliclazide and prazosin can be proven, which are currently scarcely reported.

(b) This research can also show the potential of UASB reactor in treating trace pharmaceutical compounds as an enhancement for the existing conventional WWTPs when operated at the right operational condition.

(c) The relationship between trace pharmaceutical compounds and anaerobic process performance can also be confirmed. By means, if the removal of the compounds is dependent on anaerobic parameters only, or the compounds are also able to affect the anaerobic process performance.

(d) This research can ascertain the impact of caffeine, gliclazide and prazosin at trace concentration towards process parameters and microbial diversity in an anaerobic reactor operation.

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1. **Azizan, N. A. Z.**, Kamyab, H., Yuzir, A., Abdullah, N., Kirpichnikova, I., Oryani, B., Rezania, S. (2022). 'The effects of caffeine, gliclazide, and prazosin on the performance and microbial diversity in an up-flow anaerobic sludge blanket (UASB) reactor'. *Biomass and Bioenergy*, 163, 106511. doi: https://doi.org/10.1016/j.biombioe.2022.106511. **(Q1, IF: 5.774)**
2. **Azizan, N. A. Z.**, Kamyab, H., Yuzir, A., Abdullah, N., Vasseghian, Y., Ali, I. H., Elboughdiri, N., Sohrabi, M. (2022). 'The selectivity of electron acceptors for the removal of caffeine, gliclazide, and prazosin in an up-flow anaerobic sludge blanket (UASB) reactor'. *Chemosphere*, 303, 134828. doi: https://doi.org/10.1016/j.chemosphere.2022.134828. **(Q1, IF: 8.943)**
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1. **Azizan, N. A. Z.,** Yuzir, A., Al-Qaim, F. F., and Abdullah, N. (2020). Anaerobic Treatment Performance in Presence of Pharmaceutically Active Compounds. *IOP Conference Series: Earth and Environmental Science, 479*, 012029. doi: 10.1088/1755-1315/479/1/012029. **(Indexed by SCOPUS)**