CO-DIGESTION OF PETROCHEMICAL WASTEWATER WITH ACTIVATED MANURE IN CONTINUOUS STIRRED TANK REACTOR FOR METHANE PRODUCTION

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Thesis submitted in fulfillment of the requirements
for the award of the degree of
Doctor of Philosophy (Civil Engineering)

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APRIL 2015

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This thesis is dedicated to

My beloved parents

M. A. Kasem Mian and Nurun Nahar Begum

For their endless care and comfort

&

My dear brother

Nurun Nobi

For his care, support and suggestion

ACKNOWLEDGEMENTS

I would like to express my most sincere gratitude to the supervisory committee Professor Dr. Zularisam bin Abdul Wahid for his continuing support, professional guidance and for giving me an opportunity to learn what research is all about. Special gratitude should be expressed to Senior Lecturer Dr. Anwar Ahmad for his contributions, guidance and time towards this research.

Sincerely thanks should be forwarded to Vice Chancellor University Malaysia Pahang (UMP), Profesor Dato' Dr. Daing Nasir Ibrahim for the DSS scholarship and also sponsorships to the International Conference for Postgraduate Research Studies, at Zanith hotel, Kuantan 2014.

I extend my deepest gratitude to Dr. Rezaul Karim, and Dr. Mir Sujaul Islam for their great effort and support during my study period.

Special gratitude also should be expressed to my family, especially to my father, M. A. Kasem Mian; my mother, Nurun Nahar Begum; my brother Nurun Nobi, for their patience and morale support.

Finally, I thank to all my friends especially Tanveer Ahmed Khan, Abul Hasham Beg, Khandokar Zahid Reza, and Md. Rafiqul Islam who have contributed this research.

ABSTRACT

The combined challenges of environmental crisis and declining fossil fuel supplies are driving intensive research focused on alternative energy production. Particularly, today's generation is facing two coexisting problems: the proper management of wastes generated from the industrial sectors, and the scarcity for novel resources of gasoline to meet up energy demand of civilization. Anaerobic co-digestion, a sustainable green technology, presents an outstanding opportunity for both energy conversion and pollution control. Therefore, it has become a core method treating organic wastes on account of its economic benefits of energy generation. The continuous stirred tank reactor (CSTR) can be defined as a sealed-tank digester equipped with mixing facility. Chemical pretreatment coupled with anaerobic co-digestion technology was applied on petrochemical wastewater using CSTR focusing on enhanced hydrolysis and methanogenesis. Batch experiments were performed, with applied H_2O_2 doses of 0.5%, 1% and 1.5% for contact times of 5, 10 and 15 min. Results revealed that 1% H_2O_2 dose (1.0mM Fe^{3+}) for 5 min exposure elevated biodegradability index (BOD/COD) up to 35%. Subsequently, batch experiments were employed with various mixing proportions of petrochemical wastewater (PWW): dairy cattle manure (DCM): beef cattle manure (BCM), such as 25: 37: 38, 40: 30: 30, 50: 25: 25, 60: 20: 20, and 75: 12: 13. Results revealed that PWW: DCM: BCM ratio (50: 25: 25) provided maximum methane production. Although methane production is considered to get introverted by VFA accumulation leading to reactor instability during anaerobic digestion, a 10 mg/L of NH_4HCO_3 dosing and the co-digestion of PWW together with BCM and DCM caused 50% enhancement in methane production, followed by a 98±0.5% reduction in COD at 10 days hydraulic retention time. No VFA buildup was identified. In comparison with the digestion of PWW alone, methane yield increased by 50–60% under mesophilic conditions and 50–65% under thermophilic conditions due to co-digestion. This was induced by an optimum C: N ratio (30:1) of the feed stock ensuring microbial growth and buffering capacity. The anaerobic digestion, biogas generation, and energy assessment were analyzed for ten flow rates; 170, 220, 300, 370, 410, 475, 540, 600, 640 and 680 mL/day. The analytical data revealed that the environmentally complied optimum flow rate was 170 mL/day, for maximum methane generation. As the F/M proportion varied from 0.25 to 2.0 and organic loadings from 6.31-27.14 (g VS/L), however, it has been observed that the methane yield increased from 451.9±15 to 461.5±17 and 519.8±15 to 520.9±16 mL/ g VS as the F/M ratio increased from 0.25 to 0.5 for mesophilic and thermophilic states respectively but decreased gradually even when the F/M ratio increased up to 2. However, considering all the factors F/M ratio of 0.5 was observed to be the optimum to avoid system imbalance. This work may help in minimizing the environmental issues of petrochemical wastewater treatment in the future.

ABSTRAK

Cabaran gabungan krisis alam sekitar dan penurunan bekalan bahan api fosil memacu penyelidikan intensif yang memberi tumpuan kepada pengeluaran tenaga alternatif. Terutama, generasi hari ini menghadapi dua masalah bersama: pengurusan bahan api betul bahan buangan daripada sektor perindustrian, dan kekurangan sumber untuk novel yang baru untuk memenuhi permintaan tenaga yang sesuai. Anaerobik bersama penghadaman, teknologi hijau yang mampan, menyediakan peluang yang luar biasa untuk kedua-dua penukaran tenaga dan kawalan pencemaran. Oleh itu, ia telah menjadi satu kaedah utama untuk mengolah sisa organik disebabkan manfaat ekonomi di dalam sektor tenaga. Reaktor tangki berterusan dikacau (CSTR) boleh ditakrifkan sebagai pencerna dimeteraikan tangki dilengkapi dengan kemudahan pencampuran. Praolehan kimia ditambah pula dengan teknologi anaerobik bersama penghadaman telah digunakan pada petrokimia air sisa menggunakan CSTR memberi tumpuan kepada hidrolisis dipertingkatkan dan methanogenesis. Eksperimen Batch telah dilakukan, dengan gunaan H_2O_2 dos sebanyak 0.5%, 1% dan 1.5% untuk kali kenalan 5, 10 dan 15 min. Hasil kajian menunjukkan bahawa 1% dos H_2O_2 (1.0mm Fe3 +) untuk 5 min pendedahan tinggi indeks biodegredasi (BOD / COD) sehingga 35%. Selepas itu, eksperimen kumpulan bekerja dengan pelbagai perkadaran campuran air sisa petrokimia (PWW): lembu tenusu baja (DCM): lembu daging lembu baja (BCM), seperti 25: 37: 38, 40: 30: 30, 50: 25: 25, 60: 20: 20, dan 75: 12: 13. Hasil kajian menunjukkan bahawa PWW: nisbah BCM: DCM (50: 25: 25) dengan syarat pengeluaran metana maksimum. Walaupun pengeluaran metana dianggap untuk pendiam oleh VFA pengumpulan membawa kepada reaktor ketidakstabilan semasa penghadaman anaerobik, 10 mg / L daripada NH_4HCO_3 dos dan bersama pencernaan PWW bersama-sama dengan BCM dan DCM disebabkan peningkatan 50% dalam pengeluaran metana, diikuti dengan pengurangan 98 ± 0.5% dalam permintaan oksigen kimia di 10 hari masa tahanan hidraulik. Tiada VFA penumpukan telah dikenal pasti. Dalam perbandingan dengan pencernaan PWW sahaja, hasil metana meningkat sebanyak 50-60% di bawah keadaan mesophilic dan 50-65% di bawah keadaan thermophilic kerana bersama penghadaman. Ini telah didorong oleh C optimum: nisbah N (30: 1) stok suapan memastikan pertumbuhan mikrob dan kapasiti buffering. Pencernaan anaerobik, penjanaan biogas, tenaga dan penilaian dianalisis bagi sepuluh kadar aliran; 170, 220, 300, 370, 410, 475, 540, 600, 640 dan 680 mL / hari. Data analisis menunjukkan bahawa kadar aliran optimum yang dipatuhi alam adalah 170 mL / hari, untuk generasi metana maksimum. Sebagai nisbah F / M diubah 0.25-2.0 dan beban organik 6.31-27.14 (g VS / L), walau bagaimanapun, ia telah diperhatikan bahawa hasil metana meningkat daripada 451.9 ± 15-461.5 ± 17 dan 519.8 ± 15-520.9 ± 16 mL / g VS sebagai nisbah F / M meningkat 0.25-0.5 untuk negeri mesophilic dan thermophilic masing-masing tetapi menurun secara beransur-ansur walaupun nisbah / M F meningkat sehingga 2. Walau bagaimanapun, memandangkan kesemua faktor nisbah F / M 0.5 diperhatikan untuk menjadi yang optimum untuk mengelakkan ketidakseimbangan sistem. Kerja ini boleh membantu dalam mengurangkan isu-isu alam sekitar rawatan air sisa petrokimia pada masa akan datang.

TABLE OF CONTENT

CHAPTER 1 INTRODUCTION

CHAPTER 2 LITERATURE REVIEW

CHAPTER 3 METHODOLOGY

CHAPTER 4 IMPLEMENTATION AND RESULTS

CHAPTER 5 CONCLUSION AND FUTURE WORK

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A	Cross-sectional area
ACD	Anaerobic co-digestion
AD	Anaerobic digestion
AFBR	Anaerobic fluidized bed reactor
AHMPR	Anaerobic hydrogen and methane production reactor
AM	Activated Manure
ASB	Activated sludge biomass
ASBR	Anaerobic sequencing batch reactor
ASCD	Anaerobic semi continuous digester
ATCC	American type culture collection
BCM	Beef cattle manure
BOD	Biochemical oxygen demand
BOD_5/COD	Bio-degradability index
BDOC	Biodegradable dissolved organic carbon
CA	Catalase activity
CBCTT	Chemical and biological coupled treatment technology
C/N	Carbon to nitrogen ratio
COD	Chemical oxygen demand
COD_f	Feed Chemical oxygen demand
COD_{in}	Influent chemical oxygen demand

CSTR	Continuous stirrer tank reactor
DCM	Dairy cattle manure
DOC	Dissolved organic carbon
F/M	Food to micro-organism ratio
FAFBR	Flocculant-anaerobic fluidized bed bioreactor
FSAD	Full scale anaerobic digester
HRT	Hydraulic retention time
H_2O_2	Hydrogen peroxide
IBR	Integrative biological reactor
MSW	Municipal solid waste
OLR	Organic loading rate
OHP	Oxidation by hydrogen peroxide
PWW	Petrochemical wastewater
Q	Flow rate
SD	Standard deviation
SRT	Sludge retention time
SS	Suspended solids
TKN	Total Kjeldahl nitrogen
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorous
TSS	Total suspended solids
TT	Thousand tons

UASB	Upflow anaerobic sludge blanket
UASSR	Up-flow anaerobic solid state reactor
UNFT	Unfiltered Turbidity
VFA	Volatile fatty acid
VSS	Volatile suspended solids
° C	Degree Celsius
KM	Kilometer
min	Minute
cm	Centimeter
cm^2	Square centimeter
d	Diameter
g	Gram
H	Height
h	Hour
Kg	Kilogram
L	Liter
M	Micro mole
m^3	Cubic meter
V	Velocity
µ	Micro (10^{-6})
P	Phosphorous
N	Normality

TS	Total solids
NH_4HCO_3	Ammonium bicarbonate
MFBR	Mesh filter bioreactor
SAMR	Submerged anaerobic membrane reactor
SMAR	Self-mixing anaerobic reactor
SCR	Semi continuous reactor
STP	Standard temperature and pressure

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Currently, the world is confronting two parallel issues: i) the appropriate control of waste originating from manufacturing areas and ii) the lack of innovative fuel resources to meet increasing energy requirements (Harsono et al., 2014; Tommasi et al., 2013). Simultaneous ecological battles and deteriorating fuel supplies have inspired laborious studies to improve energy reserves. The most common substitute reserve stock for fossil fuels in the petrochemical subdivision is biomass (Bustamante et al., 2013; Serrano et al., 2013). Nevertheless, surplus waste harvests can also act as distinct fossil fuel reserve stocks. Particularly, at the start of the 21st century, the world is facing environmental contingency of wastewater management and global warming due to population rise, industrialization, solid waste generation, urbanization and unplanned waste management. Integrating these into whole generates huge untreated industrial and domestic wastewater which is carcinogenic to the human beings. In particular, wastewater generated from petrochemical industries is a complex mixture of polycyclic aliphatic and aromatic petroleum hydrocarbons (Bierkens and Geerts, 2014; Ghorbanian et al., 2014; Métayer et al., 2014; Yanto and Tachibana, 2014) in which aromatic portion cannot be easily digested by commonly practiced methods. Petrochemical wastewater

(PWW) possesses much oxygen undermining potential (COD 1-60 g/L) as industrial wastewater becomes huge challenge to meet progressively strict environmental guidelines (Lakatos et al., 2014; Maretto et al., 2014; Patel and Madamwar, 2002; Shavisi et al., 2014; Wang et al., 2014a). The deficiency of wastewater management absolutely affects natural divergence of the aquatic ecosystems, disordering the elementary integrity of total ecosystems. So, the prevention of continuous pollution caused by petrochemical effluents is obligatory. Anaerobic digestion (AD) presents an outstanding opening for energy conversion and pollution minimization mutually (Alvarez et al., 2014; Sankaran et al., 2014; Zhang et al., 2014a).

The conventional treatments include gravitational separation, centrifugation, ozonation, wet oxidation, application of coagulants, flocculants, flotation, ultra filtration or sorption and advanced treatment process (Parilti, 2010; Vallejo et al., 2015; Zhuang at al., 2014). The anaerobic digestion system among all treatment options had been accepted as the fundamental one of a progressive mechanism for environmental safeguard (Siddique et al., 2014). To meet up growing requirement for energy and financially-advantageous treatment strategy, AD system has become the motivation of universal consideration (Hidalgo and Marroquín, 2014). In comparison with former technologies, the main benefits of AD system are minor sludge yield, minimum budget, great energy feedback and process stability. Besides, it provides an optimistic environmental influence accommodating waste management with net energy generation. The technology also permits the utilization of effluent as compost. Numerous researchers have studied anaerobic digestion since the last decade (Dareioti and Kornaros, 2014; Kythreotou et al., 2014; Montañés et al., 2014; Ortner et al., 2014; Theofanous et al., 2014; Vrieze et al., 2014; Wang et al., 2014b; Yu et al., 2014; Zhang et al., 2014b). Nevertheless, despite these benefits, anaerobic digestion is not practiced widely in PWW treatment due to its slow reactions, leading to long hydraulic retention time (HRT), volatile fatty acid (VFA) accumulation, and poor process stability. Hence; this study on this basis is focused.

Anaerobic digestion is accomplished via three basic mechanisms; namely hydrolysis, acidogenesis and methanogenesis (Niu et al., 2014 and Lu et al., 2013). Notable that hydrolysis is considered to be a rate-defining stage in AD; specifically, due to recalcitrant substrates. Fatty acids in wastewater have an inhibitory impact on many microorganisms, which makes biological degradation difficult (Chandra and Mohan, 2014 and Niu et al., 2013). Pretreatment might play a role in improving biochemical degradation efficiency (Wang et al., 2014c; Yu et al., 2014). Many pretreatments aspire to solubilize or hydrolyze the compounds to improve degradability in biotic reactors. Those consist of physical dimension reduction, thermal hydrolysis, ultrasonic treatment, chemical treatment by acid or alkali, ozonation and oxidation by H_2O_2. H_2O_2 is a versatile, vigorous oxidative agent that reacts via a hydroxyl radical mechanism with an oxidizing potential of 2.6V, which reduces chemical oxygen demand (COD), produces H_2O and CO_2, and enhances biodegradability of organic matters.

Even if there were widespread application of AD, the methane generation would be squat and related to elevated nitrogen and lignocellulose content (Rajagopal et al., 2013; Yenigün and Demirel, 2013). Hence, co-digestion of pretreated PWW with beef cattle manure and dairy cattle manure could offer an efficient solution, with marked reduction in volatile fatty acid (VFA) accumulation and improved reactor stability. In this study, we focused on the consequence of various mixing proportions on methane generation latent and stability of continuous stirred tank reactor under different conditions.

A CSTR can be defined as a closed-tank digester equipped with mixing facility (Diana et al., 2013; Yang et al., 2013). Mechanical instigator renders much area of contact between substrate and microorganisms thus ameliorating gas production. Over and above feeding of anaerobic digesters are amalgamated to assure competent transmission of organic compound for the active bacteriological biomass, to discharge gas bubbles grabbed in the system and to avoid precipitation of heavier coarse substance (Zhang et al., 2014c; Ward et al., 2008).

1.2 CHEMICAL PRETREATMENT AND ANAEROBIC CO-DIGESTION IN CONTINUOUS STIRRED TANK REACTOR

The application of H_2O_2 as an oxidizing auto catalyst already proved treating halogenated hydrocarbon endures in waste water treatment (Oh et al., 2014; Zhang and Li, 2014). To degrade variety of hazardous wastes in situ chemical oxidation (ISCO) has been used as an encouraging inventive technique (ITRC, 2001; ITRC, 2005). H_2O_2 oxidation mechanism might be made of a struck via OH radicals on the carbon-hydrogen chain of fatty acids. OH^- radicals possess muscular capability to breakdown the aromatic ring fixed to hydroxyl groups exists in fatty acids. These accelerate development of water-soluble complexes through cogitation of hydrogen and addition of oxygen atoms through contribution of ferrous or ferric ions. This process generates minor aliphatic compounds, resulting from infringement of lengthier hydrocarbon chains of fatty acids and lastly provokes mineralization of preliminary organic matters. In combination of biological treatment, make oxidation by hydrogen peroxide (OHP) an innovative alternative for advanced waste water treatment (Apollo et al., 2014).

Habitually, in industrial effluents, fraction of digestible COD which may be symbolized by biodegradable dissolved organic carbon (BDOC) is relatively low (Apollo et al., 2014; Tripathi et al., 2011). Hence, to enhance the treatment productivity, an evocative oxidant that enhances BDOC of raw wastewater is obligatory. H_2O_2 can transubstantiate several refractory organic compounds to biodegradable ones, i.e., BDOC that can be eliminated simply via biodegradation. Earlier researchers reported that H_2O_2 might enhance biodegradability of organic waste products producing most effective intermediates like OH^- radicals in presence of Fe^{2+} (Babu et al., 2010; Long et al., 2007; Socías et al., 2013). Scientists reported that OH radicals produced via Fenton-like mechanism are proficient of oxidation of plentiful organic matters, including diesel (Ferguson, 2004; Kong et al., 1998; Li et al., 2013; Prabir et al., 2011; Yeh et al., 2008;), chlorinated ethylenes (Yeh et al., 2003), aromatic hydrocarbons (Ahad et al., 2008; Yeh et al., 2008), 2,4-dichlorophenol (Wang et al., 2013 and Zhou et al., 2008), and 4-chlorophenol (Kozmér et al., 2014 and Zhou et al., 2008).

Catalase, an antioxidant can break down H_2O_2 into water and oxygen (Milton, 2008). To split H_2O_2 into molecular O_2 and H_2O, catalases use a two-electron transfer mechanism (Guwy et al., 1999). One unit of catalase activity corresponds to disintegration of 1 µmole of H_2O_2 per minute at standardized conditions, providing a gas flow of 11.2 µl of O_2/min at Standard temperature and pressure (STP) (Guwy et al., 1999). To neutralize H_2O_2 toxicity effect on activated microbial biomass catalase activity has been employed before anaerobic co-digestion.

Methane yield of AD system might be enriched by Co-digesting sewage sludge together with agro agricultural wastes or municipal solid waste (MSW) (Alatriste- Ferrer et al., 2014; Mondragon et al., 2006; Romano and Zhang, 2008; Solli et al., 2014; Xing et al., 2014; Zheng et al., 2014). Moreover, co-digestion of cattle manure and MSW (Borowski et al., 2014; Hartmann and Ahring, 2005; Lindmark et al., 2014) provided increased methane yield. A predominantly resilient cause for co-digestion of wastes is the appropriate fixation of carbon-to-nitrogen (C: N) ratio. The optimal C: N ratio of 25–30:1 is usually utilized by microorganisms. Nonetheless C: N ratios may frequently be significantly lesser than this, for instance the C: N ratio of sewage sludge is around 9:1 (Kizilkaya and Bayrakli, 2005). Wastewaters can differ extensively in C: N values. The two-stage reactor through biomass retention has been investigated to reflect the proficiency of dependable performance having C: N ratios less than 20 (Mata-Alvarez, 2002). The ideal C: N ratio may be achieved by co-digesting low and high C: N ratio wastewater like as biomass. In order to increase methane production compared to the conventional method co-digestion has been chosen in this study.

Temperature plays a significant role as an operational parameter for AD method (Siddique et al., 2014). The effect of temperature on bacterial growth and degradation frequency may be demonstrated by the Arrhenius equation (Batstone et al., 2002; Hao et al., 2002; Siegrist et al., 2002). AD at thermophilic conditions presents numerous benefits like enhanced reaction frequency and ameliorated bio-digestibility of organics (Kim et al., 2002; Rintala, 1997; Yu et al., 2014). Noticeable that an alteration from mesophilic to thermophilic conditions is conducted by a remarkable (over 80%) and prolonged (over 4 days) reduction in methane yield because of acclimatization of methanogens to thermophilic state (Van Lier et al., 1992; Visser et al., 1993). Nonetheless, mesophilic methanogens were exposed to bear short-range temperature rise (Speece and Kem, 1970 and Ahn and Forster, 2002) or sludge interchange between mesophilic and thermophilic digesters (Song et al., 2004). Therefore, both conditions were executed to study the performance of CSTR.

This study also proposes the application of ammonium bicarbonate (NH_4HCO_3), due to its buffering capability opposite to acidity throughout operational period and also to maintain bacteriological population balance. Therefore, significant roles will be performed by NH_4^+ as the recommended microbial nutrient for nitrogen and buffering capability in an anaerobic reactor (Gerardi, 2003). Nonetheless, excessive NH_4HCO_3 concentrations create free ammonia toxicity particularly for methanogensis (Niu et al., 2013; Sawayama et al., 2004). Hence, the optimal dosage for NH_4HCO_3 applied as supplementation in AD system should be investigated.

The co-digestion of petrochemical wastewater with activated manure has not been widely examined; the abrupt pH drops resulting in reactor failure and volatile fatty acid (VFA) accretion have not been studied, nor have the optimal ratios for CH_4 production. Furthermore, no previous studies on the simultaneous investigation of ecological, energetic and financial features have been published. Consequently, the work presented here may help lessen the environmental issues related to petrochemical manufacturing. This research focuses on the environmental, energy-related, and fiscal potential of anaerobic co-digestion (ACD) of PWW with AM in a CSTR operated in thermophilic conditions. Laboratory-scale trials examining COD elimination ability, the rank of treated samples and the optimal CH_4 yield under distinct waste blends (100 % PWW; 90 % PWW/10 % AM; 80 % PWW/20 % AM; 70 % PWW/30 % AM; 60 % PWW/40 % AM; 50 % PWW/50 % AM; 40 % PWW/60 % AM; 30 % PWW/70 % AM; 20 % PWW/80 % AM; 10 % PWW/90 % AM; 100 % AM) are explained throughout the text.

Although anaerobic bio-digestion is being extensively applied to the industrial treatment plants, the optimum flow rate to feed the bio-digester still remains as the burning question. Consequently, our research motivation was to Figure out the optimum flow rate that optimized both biogas generation and bio-digestion. Incoming wastewater must flow through a treatment plant at a rate that allows microorganisms sufficient time to consume the incoming food and to settle properly. High flows can shorten the time necessary for the full treatment of wastewater. Extremely high flows can wash microorganisms out of the plant through the final clarifier. There is an obvious influence of influent flow rate on the biogas generation during anaerobic process. It shows a vast research gap in determining the optimal flow rate during the industrial application of the bioreactors. Moreover, being confused about the optimal flow rate during reactor operation represents a huge energy loss taking place every year in the industrial sector. A definite flow rate has become increasingly important to comply with the increasingly stringent environmental regulations by providing adequate treatment of effluents from industrial sources. Therefore, to determine the influence of feed flow rate on the biogas generation and the degradation of wastewaters from the petroleum refinery at Terengganu, Malaysia using a continuous CSTR-type bio-digester was one of the key objectives of this present study.

Incoming wastewater to a treatment plant provides the food that microorganisms need for their growth and reproduction. This food is mostly organic material. The more soluble the organic material is, the more easily microorganisms can use it. Therefore, the optimum food to microbe ratio to operate the CSTR still remains unknown. In particular, the influence of the F/M proportion on the anaerobic co-digestion of PWW and activated manure operating under both mesophilic and thermophilic conditions has not been studied extensively. Consequently, our research motivation was to investigate the effect of F/M proportion, which optimized both biogas generation and bio-digestion. Therefore, the principal objective of this work was to observe the influence of F/M proportions (0.25 to 2.0) on biogas generation and organic pollutants reduction from PWW under mesophilic and thermophilic states.

1.3 PROBLEM STATEMENT

Anaerobic digestion is being used effectively in numerous fields such as petrochemical industries, POME industries, distillery industries, olive-oil industries, piggery wastewater, dairy wastewater, fishery wastewater, municipal wastewater, and slaughterhouse wastewater to protect environmental pollution as well as to generate energy (Latif et al., 2011). Particularly for petrochemical wastewater, investigations reported that aldehydes, acids, alcohols, and esters might be employed for methane yield after lengthy acclimatization (Siddique et al., 2014). The existence of hydroxyl groups and a growing carbon chain decreased the toxicity of complexes to the digester microflora. Adaptations to aromatic ring and double-bond compounds are time consuming. The most common design applied in AD is continuous stirred tank reactor. The major problem of the reactor is prolonged retention time and minor gas yield. Presently, anaerobic digestion is facing following impairments:

[1] sluggish reactions, that need lengthier HRT and indigent system stability in commonly practiced designs

[2] operational failure is caused by an abrupt drop of pH & accumulative concentration of VFA

[3] insufficient buffering control & distraction of bacterial population stability between non-methanogen & methanogen to transform carbonaceous organic to CH_4

[4] sludge sustaining problem with multi-chamber fixed film anaerobic treatment remained unsolved

[5] sludge washed out from the anaerobic up-flow fixed-film reactor

[6] lower COD removal by anaerobic hybrid reactor therefore, pollution control with energy production strategy of anaerobic digestion process still facing stability challenge and retention time challenge.

1.4 OBJECTIVES OF RESEARCH

This research focuses on the anaerobic co-digestion of petrochemical wastewater with beef and dairy cattle manure in CSTR having chemical pretreatment strategy. Anaerobic co-digestion is proposed based on the biodegradation technique to avoid slow reaction, prolonged startup, volatile fatty acid accumulation, reactor failure, sludge washout and to enhance methane production capability of CSTR. This research framework combines chemical and biological treatment system much effectively. It can be applied in petrochemical industries, POME industries, distillery industries, olive-oil industries, slaughterhouse wastewater etc. Therefore, it meets up the energy demand cost effectively.

The objectives of this research are projected below:

i. To characterize petrochemical wastewater and determine the optimal H_2O_2 pretreatment dosing before anaerobic co-digestion of petrochemical wastewater and cattle manure in continuous stirred tank reactor for COD reduction and enhanced biodegradability

ii. To determine the optimal mixing proportion of Petrochemical wastewater and cattle manure during anaerobic co-digestion in continuous stirred tank reactor for COD reduction and enhanced bio-methane production

iii. To determine the optimal flow rate during anaerobic co-digestion of Petrochemical wastewater and cattle manure in continuous stirred tank reactor for COD reduction and enhanced bio-methane production

iv. To determine the optimal food to microbe ratio (F/M) during anaerobic co-digestion of Petrochemical wastewater and cattle manure in continuous stirred tank reactor for COD reduction and enhanced bio-methane production

v. To determine the optimal ammonium bicarbonate dosing and to reduce the VFA accumulation during anaerobic co-digestion of petrochemical wastewater and cattle manure using continuous stirred tank reactor for system stability

1.5 SCOPES OF RESEARCH

The scopes of this research are as follows:

i. Application of chemical & biological coupled treatment technology which will investigate effective H_2O_2 dosing, enhancement in BODC, catalase activity activation neutralizing H_2O_2 toxicity effect prior to anaerobic digestion, enhancement of biodegradability index, COD & TOC removal achievement.
ii. Application of co-digestion method which will investigate the balance of nutrients at an appropriate C: N ratio and a stable pH necessary for enhanced CH_4 yield the effect of temperature and organic loading rate on the rate of CH_4 generation & reduce HRT, reduction in VFA accumulation.
iii. Application of supplementation of ammonium bicarbonates which will investigate the optimal NH_4^+ dosing ensuring the process stability of anaerobic digestion enhancing CH_4 production as well.

1.6 ORGANIZATION OF THESIS

The current research has been organized to provide features on the particulars, observations, logics, interpretations and ways to meet the objectives. Chapter 1 commonly provides the momentary background of anaerobic degradation, problem statement, objectives and scope of the research. Chapter 2 describes the literature review of H_2O_2 pretreatment, biodegradable dissolved organic carbon formation, catalase activity, anaerobic degradation and anaerobic co-digestion. Chapter 3 explains the framework of the proposed chemical and biological coupled treatment technology (CBCTT). Different stages and mechanism of the same and definition of the notation have been described. Chapter 4 reports the implementation of CBCTT framework and compares the performance with other techniques. The conclusions of current research are summarized in Chapter 5. Ideas and recommendations for future work have also been projected in this chapter.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

This chapter brings out a short review on fundamental concept of chemical and biological coupled treatment technology treating petrochemical wastewater. The review covers characterization of petrochemical wastewater, effect of chemical pretreatment and catalase activity, working principle of anaerobic digestion, different types of anaerobic reactor setup for enhanced biogas production, assessment of reactor performance, investigation of factors affecting system off-set and model development on anaerobic co-digestion process. The need of pretreatment and working principle of anaerobic digestion is obligatory to understand the back ground of anaerobic system. The contribution of catalase activity is a must to bridge the gap between chemical and biological combined treatment technology. In case of anaerobic reactor setup, emphasis will be provided to continuously stirred tank reactor (CSTR) and high rate anaerobic reactors with their benefits and limitations. This will explain the reason why chemical and biological treatment has been coupled together. Investigation of factors affecting system off-set will highlight the effect of temperature, organic loading, hydraulic retention time, VFA accumulation, pH, C: N ratio. This investigation will help a lot to analyze the experimental out comes. An abridged review on model construction of chemical and biological coupled treatment technology will illustrate a fundamental overview for the construction of mathematical modeling projected in this research.

2.2 PROPERTIES OF PETROCHEMICAL WASTEWATER

Malaysia has the world's 23rd crude oil reserves inclusive condensates (5.52 billion barrels). Malaysia also possesses the world's 14th natural gas reserves (14.66 billion barrels). The 2010 projected hazardous waste generation per annum of particular ASEAN Countries is provided in Figure 2.1.

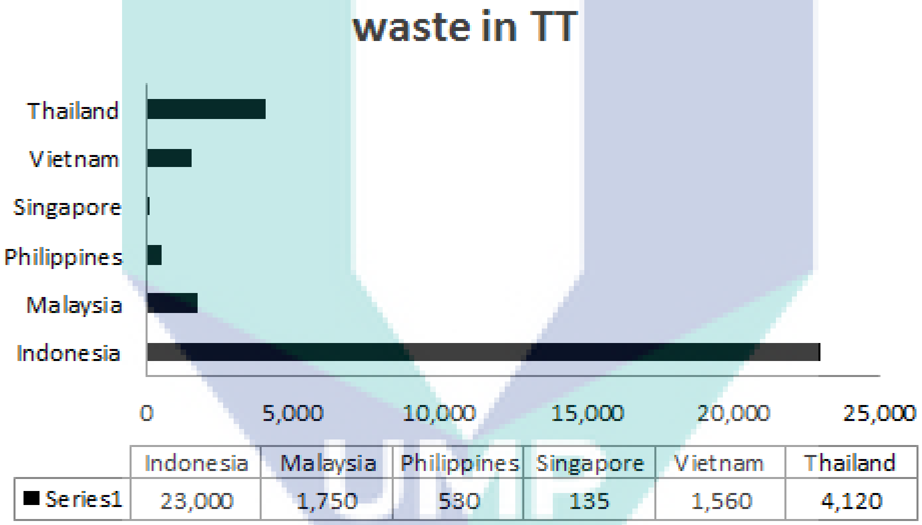

Figure 2.1: 2010 projected hazardous waste generations per annum of particular ASEAN Countries (source: Hernandez, 1993; UNEP, 1994; United Nations, 1995 and Nelson, 1997).

Malaysia stated approximately 345 thousand metric tons (TT) of hazardous wastes accumulation in 2000. Out of that particularly, 24 % comes from chemical, 6% comes from gas industry (UNEP, 2002). Organization of Petroleum Exporting Countries (OPEC, 2011) reported that, energy needs has risen from 55 million barrels of oil equivalent/day (mboe/day) in 1960 to 227 mboe/day in 2008. Energy needs will be increasing with economic expansion, the world population growth, and living standards improvement.

Wastewater discharged from petroleum refinery comprised of excessive aliphatic and aromatic petroleum hydrocarbons that might cause vigorous pollution on earth and water bodies (Dsikowitzky et al. 2004a; Dsikowitzky et al. 2004b; Shahrezaei et al. 2012 and Yanto and Tachibana, 2014). Refineries produce contaminated effluent, comprising COD concentration about 11000 mg/L, pH ranges between 2.5-8.75, total fatty acid concentration approximately 9000 mg/L comprising (m/v) acetic acid- 70%, propanoic acid- 16%, isobutyric acid- 2.5%, n-butyric acid- 7-9%, iso-valaric acid- 1%, n-valaric acid- 2-3% (Britz et al., 1983; Patel and Madamwar, 2001; Patel and Madamwar, 2002; Parilti, 2010). Previous researchers reported physic-chemical properties of petrochemical wastewater as projected below (Table 2.1):

Table 2.1: Physico-chemical characteristics of PWW

Parameter*	**Patel and Madamwar, 2002**	**Gasim et al. (2010)**
pH	2.5-2.7	6
BOD	30-32	-
COD	50-60	1.06
TS	0.02-0.3	0.19[a]
SS	-	0.06[a]
TN	0.05-0.212	0.023
Oil and Grease	0.012-0.013	-

All parameters are in g/L except pH

The amount and properties of effluent produced depend on the system conFigureuration (Shahrezaei et al., 2012).

2.2.1 Environmental Regulations of PWW Discharge

According to Environmental Quality Act, 1974 effluent discharge standards generally permeable petrochemical wastewaters are projected in Table 2.2.

Table 2.2: Permit able limits of industrial release of standards A and B (Environmental Quality Act, 1974, 1974)

Parameter*	**EIA Standard A(2009)**	**EIA Standard B(2009)**
pH	6-9	5.5-9
BOD	20	40
COD	120	200
TS	-	-
SS	50	100
N	10	20
Oil and Grease	1	10

All parameters are in mg/L except pH

Standard A is suitable for releases to any inland waters inside catchment areas scheduled in 3rd Schedule, while Standard B is suitable for other inland waters or Malaysian waters.

2.2.2 Renewable Energy from Petrochemical Industry in Malaysia and World Exposure

The civilization will need minimization of reliance on fossil fuels and reducing of pollution which is induced (Largus et al., 2004). Generally, Malaysia's energy resources principally consist of oil, natural gas, hydropower and coal; however renewable energy resources such as solar energy and biomass are now being explored. It was reported that petrochemical plants have potential of producing in excess of USD 500,000/y of methane alone from anaerobic treatment (Ren, 2009). The basic substitutive feedstock of gasoline in petrochemical segment is probably to be biomass (Gosh et al., 2006). Biomass is the extensively used renewable energy resource (IEA Bioenergy, 2009). The estimation of prospective role of biomass for 2050 is intensely profound for biomass trading (UNIDO, 2012). The potentiality of biomass in industry is projected to be 18.3 EJ/year; in case of liquid markets is projected to be 30.3 EJ/year (Figures 2.2) (UNIDO, 2012).

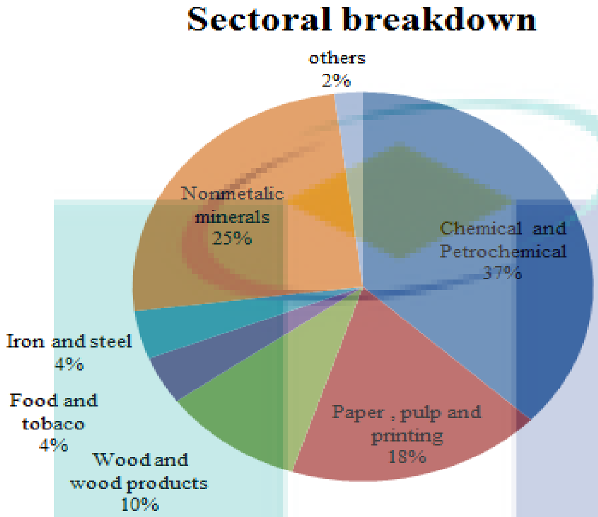

Figure 2.2: Divisional analysis of biomass latent for 2050, without interregional trade.

2.2.3 Petrochemical wastewater treatment process

The rapid growth of petrochemical industry, operation and severe accidents during transportations, treatment and disposal of wastewater has been recognized as the momentous source of environmental pollution (Métayer et al., 2014 and Parilti, 2010). Additionally, biodegradation of the hydrocarbons by naturally occurring bacteria has been described to be the core process serving in the purification of the hydro-carbon contaminated environments (Yanto and Tachibana 2014 and Challain et al., 2004). Biological degradation with pretreatment of oil/water separation is being used by the petrochemical industries to treat the wastewater generated (Ghorbanian et al., 2014 and Khaing et al., 2010). A typical breakdown of petrochemical wastewater treatment technology is projected in Figure 2.3.

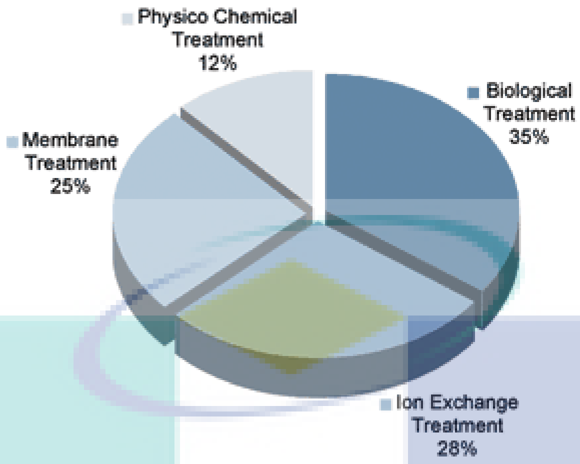

Figure 2.3: Breakdown of wastewater treatment equipment in petrochemical industry (United States 2004).

Through the former decades, numerous cost-effective treatment technics including anaerobic, aerobic and facultative processes have been established treating industrial wastewater (Latif et al., 2011). Nevertheless, strict environmental legislations and growing wants for recycling of processed water have created interests in petrochemical wastewater treatment by advanced processes (Ravanchi et al., 2009 and Vallejo et al., 2014). An extensive quantity of alternative energy recycling options exists (Brehmer et al., 2009). As the concern of climate change became challenging, there is distant encouragement for the restoration of fossil fuels by CO_2 neutral resources to attain a comprehensive deduction of greenhouse gas emissions (GHG) (BBC, 2007). In spite of these benefits anaerobic digestion of petrochemical wastewater is facing some unsolved mitigations including slow reaction, prolonged HRT, VFA accumulation and process instability.

2.3 CHEMICAL AND BIOLOGICAL COUPLED TREATMENT TECHNOLOGY

The measure of biodegradability of wastewater is expressed by biodegradability index (BOD_5/COD). Earlier research works suggested that biodegradability index should sustain in between the range of 0.4 to 0.8 in case of any wastewater to be treated by subsequent biological degradation (Aboulhassan et al., 2008). The biodegradability index of petrochemical wastewater was 0.51 initially; therefore, a chemical pretreatment was taken into account to enhance biodegradability.

Additionally, investigations explored that specifically in case of recalcitrant substrates; hydrolysis is a rate-defining stage in anaerobic digestion (Vavilin et al., 2008). To enhance biochemical degradation competence of numerous refractory materials, a quantity of pretreatment might be helpful (Wang et al., 2014a and Tripathi et al., 2011). Such pretreatments can transpose various refractory organic matters to biodegradable organics, which can be easily degraded afterwards in biological reactor (Siedlecka et al., 2005 and Zhang and Li, 2014). Several pretreatments have been performed including thermal hydrolysis, UV treatment, particle size diminution, ultrasonic effect, ozonation and OHP. Oxidation by Hydrogen peroxide (OHP), a defensible technology appeared to meet the cavity between treatment techniques of substrates with medium to rich refractory organic matter content and squat digestibility (Augustina and Vareek, 2005). Additionally, this method is apposite to treat all kinds of organic wastewater (Chamarro et al., 2001). OHP is principally interesting for petrochemical plants, production of dyes, fine and general chemical manufacturing plants, plastics, rubbers and similar industries, in addition to other low biodegradable wastewater, like landfill leachate and wastewater form waste processing plants (Volk et al., 1996).

The reaction between H_2O_2 and Fe^{2+} generates a non-selective resilient oxidant known as hydroxyl radical. The reaction pathway is expressed below:

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + \cdot OH + OH^- \qquad (2.1)$$

The reactions creating oxidizing species basically accountable for the absolute attack on organic carbon can be shown as follows:

$$RH + \cdot OH \rightarrow \cdot R + H_2O \qquad (2.2)$$

$$\cdot R + O_2 \rightarrow \cdot ROO \qquad (2.3)$$

$$\cdot ROO + RH \rightarrow ROOH + \cdot R \qquad (2.4)$$

Oxidation by H_2O_2 reaction mechanism produces OH radicals with an oxidizing potential of 2.6 V (Siedlecka et al., 2005).

Dissolved organic carbon (DOC) can be defined as a subsidiary food, assisting development of microbes and acts significant part in global carbon cycle via bacterial loop (Kirchman et al., 1991). Furthermore it is a pointer of organic loadings, also assisting terrestrial processing of organic substances. DOC is comprised of a giant fraction of biodegradable dissolved organic carbon (BDOC) in first order streams compared to higher order streams (Richard, 2010). Base flow concentrations of DOC in uninterrupted watersheds generally range between approximately 1 to 20 mg/L carbon, in absenteeism of inclusive wetlands, bogs, or swamps (Kirchman et al., 1991).

Heterotrophic bacteria might utilize organic molecules as a resource of energy and carbon that originates from BDOC fraction (Tripathi et al., 2010). Particular subset of DOC organizes the ancestors of disinfection consequences for drinking water. BDOC can underwrite to unwanted biological regrowth inside water distribution systems (Richard, 2010).

Catalase is a mutual enzyme observed in almost all living organisms incur to oxygen. It mobilizes the breakdown of H_2O_2 to water and oxygen (Chelikani et al., 2004). It is a unique enzyme in reproductive reactions. Similarly, catalase possess one of the maximum turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second (Goodsell et al., 2004). It comprises four porphyrin heme (iron) groups that let the enzyme to react with H_2O_2.

How catalase decomposes of hydrogen peroxide can be explained as follows:

$$2\,H_2O_2 \rightarrow 2\,H_2O + O_2 \qquad (2.5)$$

The existence of catalase in a microbial or tissue sample can be checked by the addition of a volume of hydrogen peroxide and observation of the reaction. The accumulation of bubbles, oxygen, specifies a positive result. As catalase shows a vigorous specific activity producing a detectable response, this simple assay can be made possible by the observation of naked eye.

Catalase are also capable to catalyze the oxidation, by hydrogen peroxide, of numerous metabolites and toxins, comprising formaldehyde, formic acid, phenols, acetaldehyde and alcohols. It can be demonstrated by following reaction:

$$H_2O_2 + H_2R \rightarrow 2H_2O + R \qquad (2.6)$$

H_2O_2 is an injurious repercussion of various ordinary metabolic processes: to save cells and tissues from damage, it must be immediately transformed into another substance. At this point, catalase is repeatedly used by cells to swiftly catalyze the breakdown of H_2O_2 into minor reactive gaseous O_2 and H_2O molecules (Gaetani et al., 1996; Kurimoto et al., 2014).

Catalase is habitually found in a cellular, bipolar environment organelle known as the peroxisome (Alberts et al., 2002). Peroxisomes take part in photorespiration and symbiotic nitrogen fixation in plant cells. H_2O_2 is employed as a powerful antimicrobial agent while cells are affected by a pathogen. Catalase-positive pathogens enable catalase to neutralize the peroxide radicals, therefore permitting them to endure uninjured within the host (Srinivasa et al., 2003).

Anaerobic digestion is the digestion of complex organic compounds under oxygen free environment (Pain and Hepherd, 1985; Yu et al., 2014; Ortner et al., 2014 and Zhang et al., 2014a). The whole process is time absorbing as microbial consortia are accountable for degradation. System consumes time to acclimatize to the new environment earlier they start to utilize organic compounds to propagate. It has been applying to an extensive range of feed-stocks such as industrial and municipal waste waters, agricultural, food wastes and plant residues. The generation of biogas by anaerobic process presents substantial benefits compared to other methods, comprising:

i. Fewer biomass sludge is generated compared to aerobic method
ii. Effective for treatment of wet wastes of less than 40% dry matter (Mata-Alvarez, 2002)
iii. Adequate pathogen removal (Bendixen, 1994; Lund et al., 1996 and Sahlsstrom, 2003). It is especially true for multi-stage digesters (Kunte et al., 2004, and Sahlstrom, 2003)
iv. Negligible odor emissions due to 99% of volatile compounds are oxidatively decomposed upon combustion (Smet et al., 1999)
v. Great compliance with various national waste policies implemented to decrease the volume of biodegradable waste incoming land fill.
vi. The digestate produced is an enriched fertilizer for both its handiness to plants (Tafdrup, 1995) and its rheology (Pain and Hepherd, 1985).
vii. An option of carbon impartial energy is generated as biogas.

However, the fundamental stages of anaerobic degradation are projected below (Figure 2.4): (Zeikus, 1980; Kleinstreuer and Powegha, 1982; Gujer and Zehnder, 1983 and Babu et al., 2010). Controlled anaerobic fermentation of biomass produces a gas that can be used to produce electrical–thermal energy on account of its high percentage of methane.

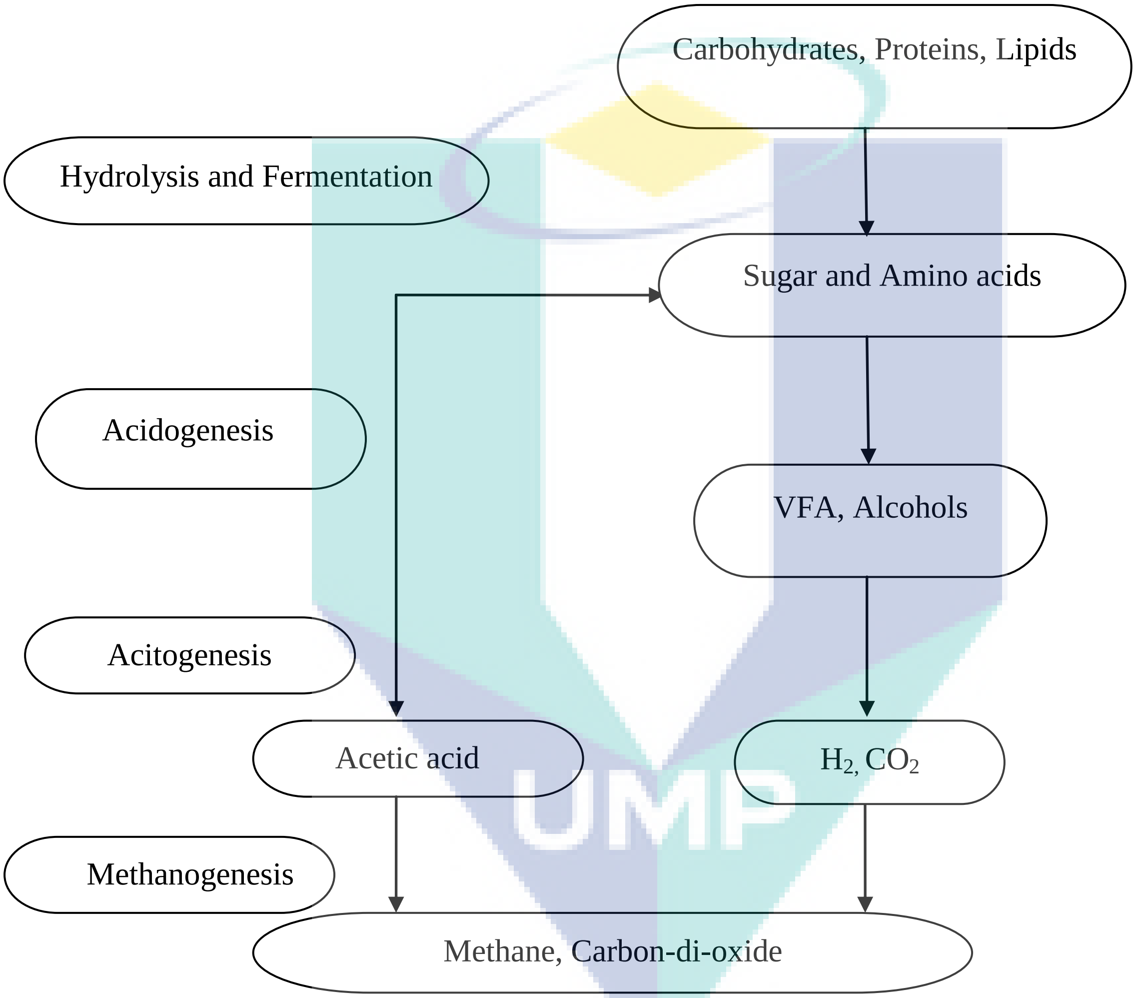

Figure 2.4: Fundamental stages of anaerobic digestion process (Babu et al., 2010; Gujer and Zehnder, 1983; Kleinstreuer and powegha, 1982; Zeikus, 1980)

2.3.1 Hydrolysis

The initial phase for digestion process is known as hydrolysis. In this phase composite organic molecules (carbohydrates, proteins, and lipids) are transformed into soluble compounds (sugar, amino-acids) that can be successively exploited by fermentative microorganisms. The set of bacteria accountable for fermentation comprises of facultative and obligate anaerobic bacteria (Metcalf and Eddy, 2003). Even though biopolymers are almost easily degradable, the cellulose of greatly lignified material is resilient to hydrolysis (Lynd et al., 2002). The rates of hydrolysis rely upon factors such as pH, temperature, constitution of organic matter and particle size of substrate (Veeken et al., 2000). Both cellulose and hemicellulose polymers are fabricated by lengthy sugar chains. After hydrolysis pretreatment they can be transformed into transitional products. Finally, they can be converted to biofuels (Öztürk et al., 2010).

2.3.2 Acidogenesis

The second phase of an anaerobic digestion process is fermentation (also known as acidogenesis). During this process further degradation of amino acids, sugars, and fatty acids takes place. The organic feedstock acts as both electron donors and acceptors. Major outcomes of fermentation are acetate, propionate, butyrate, hydrogen and carbon dioxide. Propionate and butyrate are converted to H, CO_2, and acetate. The free energy exchange related to transformation of propionate and butyrate to acetate and H_2 needs lower H_2 in process; alternatively reaction will not continue (Tchobanoglous et al., 2003).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \tag{2.7}$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \tag{2.8}$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \tag{2.9}$$

The first reaction (Equation 2.7) is much desired that produces acetic acid as the principal originator of methane. The subsequent two reactions (Equation 2.8 and 2.9) take place while agglomeration of hydrogen in process. From Equation 2.8, obvious consumption of hydrogen can be observed, while in Equation 2.9, hydrogen production is seemed to be less. The enhancement of acid load is also minor.

2.3.3 Acetogenesis

Propionate and butyrate are converted to acetate only by syntrophic acetogens (propionate and butyrate react with water and produce heat) in performance with hydrogen-utilizing methanogens (Kosaka et al., 2006, and Tatara et al., 2008). Propionate-oxidizing microbes have been acknowledged in microbes in close connotation with methanogens (Bok et al., 2004). Those microorganisms are accountable for transforming organic matters by fermentative microbial activity (Rittmann and McCarty, 2001) as shown in equations 2.10 to 2.13.

$$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2 \qquad (2.10)$$

$$CH_3COO^- + H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2 \qquad (2.11)$$

$$CH_3CH_2CH_2OO^- \rightarrow 2CH_3COO^- + H^+ + 2H_2 \qquad (2.12)$$

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \qquad (2.13)$$

Acetate is the key intermediate byproduct during bioconversion of organic substance into CH_4 and CO_2. Noticeable that approximately 70% of total CH_4 generated in AD emerge from acetate. Thereupon, generation of CH_4 from acetate is a vital phase in the AD system (Rittmann and McCarty, 2001).

2.3.4 Methanogenesis

Methanogenesis is accomplished by a set of microbes communally recognized as methanogens. Two groups of methanogenic microbes are implicate in CH_4 yield. One is known as aceticlastic methanogens which transform the acetate into CH_4 and CO_2. At the same time, the second is known as H_2 utilizing methanogens that utilize H_2 to generate CH_4. Furthermore, acetogens, are also capable of using carbon dioxide in order to oxidize H_2 and thus producing acetic acid. Nevertheless, acetic acid will be ultimately transformed into CH_4. So, the effect of this reaction is negligible. The conversion of these compounds to CH_4 is shown in equations 2.14 to 2.16 (MetCalf and Eddy, 2003):

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad (2.14)$$

$$HCO_3^- + 4H_2 + H^+ \rightarrow CH_4 + 3H_2O \quad (2.15)$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (2.16)$$

2.4 HIGH RATE ANAEROBIC REACTORS

Entire contemporary high rate AD system is established on the phenomenon of reserving huge biomass by microbial sludge immobilization. Numerous bioreactors are in action digesting waste to energy. The reactor that employed commonly is known as continuously stirred tank reactor (CSTR). Table 2.3 and 2.4 describes numerous bioreactors used for AD of various organic wastes and benefits and drawbacks of different AD reactors. From the tables2.3 and 2.4, it can be concluded that the CSTR are the simplest in terms of construction, feeding operation and cleaning.

Patel and Madamwar, 2001 anticipated an approach for acidic petrochemical wastewater treatment in single and multi-chamber fixed film anaerobic reactor. The objective was to compare the performance of fixed film anaerobic reactor with single and multi-chamber strategies. They separated acidogenesis from methanogenesis in order to achieve better project stability and increased biogas production. But ultimately their approach outstripped in a real sense due to high volatile fatty acid (VFA) accumulation, process instability and low methane production. Moreover, multi-chamber strategy was not successful treating petrochemical wastewater. Single stage strategy proved better performance over that of multi-chamber in terms of COD removal and CH_4 production (Patel and Madamwar, 2001).

Patel and Madamwar, 2002 introduced an approach treating petrochemical wastewater in anaerobic up-flow fixed-film reactor. The goal of their method was to investigate the effects of temperatures and organic loading rates on bio-methane production of petrochemical wastewater. They selected temperature range from mesophilic to thermophilic and organic loading rate from 3.60-27.20 kg COD/ m^3 d. It took a prolonged startup period of 80 days. However, this strategy achieved 98% COD removal and 0.37 m^3/ kg COD d at 15 days HRT. Then fact was that at organic loading rate of only 6.66 kg COD/ m^3 d the total system was failed due to vigorous VFA accumulation.

In 2006, Jafarzadeh et al. studied petrochemical wastewater treatment in anaerobic hybrid reactor. Two stage operations in mesophilic condition were implemented. At the end of 39 weeks when the acclimatization of the microbs to the petrochemical wastewater was done, COD reduction achievement was only 70% at HRT of 18 days and OLR of 2 kg/ m^3 d.

2.4.1 Continuous Stirred Tank Reactor

Continuous stirred tank reactor works on the principle of medium rate anaerobic system and it is still widely used for anaerobic digestion (Zhang et al., 2006). The reasons of medium rate application are the simplicity of the design system as well as independence of biomass type. To intensify this simple technology and maintain a viable population of the slow growing methanogens, the CSTRs are usually combined with an internal or external biomass separation and recycling system.

Numerous solid waste portions can also be treated in CSTRs after slurring with liquid. In CSTR feeding frequency has to be uninterrupted in order to attain extreme performance; however due to real reason reactor is fed discontinuously; mostly used rate of feeding is one time/ day (Gunaseelan, 1997). Mixing yields better interaction between microorganisms and substrates, decreases hindrance to mass transmission, reduces accumulation of resistant intermediates and balances required conditions (Leslie Grady et al., 1999). Inefficient mixing makes overall performance impaired (Stafford, 1982). Mixing might be done via mechanical device, biogas or slurry recirculation (Karim et al., 2005a). Notable that mixing enhanced efficiency of reactors treating vigorous waste (Karim et al., 2005b) whereas slurry recirculation proved improved outcomes compared to others (Karim et al., 2005c). Mixing also enhanced gas generation in comparison with unmixed reactors (Karim et al., 2005b). Slow mixing is beneficial over strong mixing (Kaparaju et al., 2008, and Stafford, 1982), for large-scale application (Stafford, 1982). The schematic of CSTR is displayed in Figure 2.5.

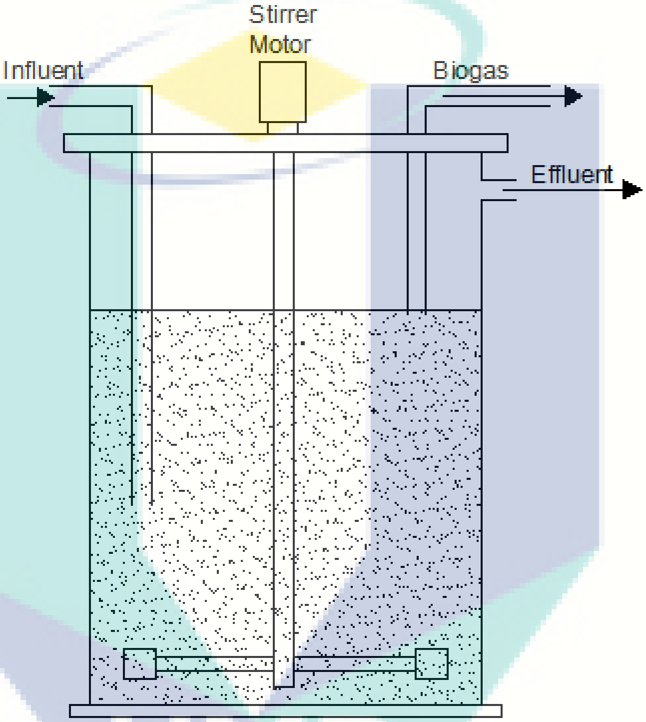

Figure 2.5: Continuous stirred tank reactor (Stafford, 1982)

Table 2.3: Bioreactors and their anaerobic digestion performance

Bioreactor type	**Type of substrate**	**Organic loading rate ($kg/m^3/d$)**	**Comments**	**References**
ASBR	Fruit and vegetable waste and abattoir wastewater	2.6	A reduction in biogas yield was found due to high ammonia accumulation at high organic loading rate.	Bouallgui et al. (2009b)
CSTR	Municipal solid waste	15	This reactor exhibited better efficiency as the OLR gradually increased up to 15 $kg/m^3/d$.	Angelidaki et al. (2006)
FSAD	Industrial food waste	17	CH_4 production from 360 l/kg waste at 40 days retention time was carried out.	Ike et al. (2010)
IBR	Kitchen waste	8	Exhibited better efficiency and biogas production rate was greater than the single reactor.	Guo et al. (2011)
SCR-Laboratory scale	Municipal solid waste and press water from municipal	20	Reactor performance for biogas yield was superior up to 20 OLR but subsequent enhancement in OLR did not affect the same.	Nayono et al. (2010)
FAFBR-starch based	Primary treated sewage effluent	-	The performance and biological activity at high OLR was better than commonly used one.	Xing et al. (2010)
MFBR-rotating drum	Municipal solid waste	15	This reactor was ascertained to be steady and useful during mixing at high OLR.	Walker et al. (2009)
SMAR	Poultry litter	16	Self-mixing at high OLR and high bio-methanation from poultry litter was found.	Rao et al. (2011)
SAMR	Sewage sludge, food waste	1.8	Unstable at beginning, but stable after acclimatization.	Jeong et al. (2010)
ASCD-self mixing	Olive mill wastewater and olive mill solid waste	14	The best performance was found through CH_4 yield, SCOD and phenol degradation and effluent quality.	Fezzani and Cheikh (2010)
AHMPR-two phase	Organic waste	3	11% higher energy was produced than single-stage reactor.	Luo et al. (2011)
UASSR	Mixture of maize silage and straw	17	USSR exposed the best Methanogenic efficiency for degradation of solid Biomass.	Mumme et al. (2010)

Table 2.4: Benefits and drawbacks of different AD reactors

Methods	**Advantages**	**Drawbacks**	**References**
UASFF	Higher OLR attainable than operating UASB Complications of clogging was removed Higher biomass retention	Granulation inhibition at huge VFA accumulation Minor OLR while treating suspended solid wastewaters	Ayati and Ganjidoust (2006)
CSTR	Provides more contact of wastewater with biomass through mixing Increased gas production compared to conventional method	Less efficient gas production at high treatment volume Less biomass retention	Lyberatos et al. (1999)
Fluidized bed	Condensed among all high-rate system Appreciable mixing capacity Vast surface area for biomass attachment	Vigorous power prerequisite for bed fluidization Huge charge of carrier media Non-applicable in high suspended solid wastewaters Usually unable of capturing produced biogas	Leslie Grady et al. (1999)
UASB	Advantageous for treating high suspended solid wastewater Capable of yielding better quality effluent Media requirement nil (less costly) High biomass retention High CH_4 yield	Reactor efficiency reliant on sludge settle ability Foaming and sludge floatation at high OLRs Elongated start-up period while granulation seed sludge is not used Granule formation inhibited at vigorous VFA agglomeration	Lettinga (1995); Kalyuzhnyi et al. (1998) and Goodwin et al. (1992)
Anaerobic contact process	Attains steady state swiftly	Stability is minor because of oxygen transfer in digester	Hamdi and Garcia (1991)

2.5 REACTOR FAILURE SYNOPSIS

During anaerobic operation organic waste is degraded to VFA, principally acetic, propionic and butyric acids (Tatara et al., 2008). Commonly, acetic acid is the major VFA in the process followed by propionic and butyric acids (Toerien and Hattingh, 1969). Indeed, anaerobic technology is related to environmental variations. VFA accumulation takes place due to deteriorations such as organic or hydraulic over loadings. The sluggish growth of H_2 consuming methanogenic bacteria is responsible for this. Inversely, the growth of glucose fermenting bacteria is fast enough producing H_2 accumulation. Frequently, it leads to reactor failure and sludge washes out.

Vigorous H_2 concentrations accelerate the accumulation of acetate, propionate and butyrate while H_2 pressure of less than 10 pa favor the production of CO_2 and CH_4 (Schmidt and Ahring, 1993; Molleta et al., 1994, and Strong and Chord-Ruwisch, 1994). It is recognized that propionic acid cannot be directly transformed to methane by aceticlastic methanogenic bacteria. It is supposed to be fragmented in to acetic acid via acetogenic bacteria. Throughout this phase it is a must to keep the H_2 concentrations to a minimum. Kaspar and Wuhrmann, 1978 reported that the optimum H_2 concentrations for propionic acid degradation are between 500-50000 ppm. Moreover, Mosley in 1983 reported that a huge concentration of H_2 to 670 ppm caused 50% reduction in the rate of propionic acid conversion.

However, reactor failure can be avoided by scholastic monitoring of some indispensable intermediate products given below:

2.5.1 Organic overloading

Organic overloading habitually arise handling concentrated wastes comprising simply degradable substrates (lactose, starch and sucrose). Abrupt discrepancy in waste composition can cause imbalance between bacterial activities in reactor that is acetogenesis runs faster than methanogenesis (Eng et al., 1986). It causes an enhancement in H_2 partial pressure and consequently a development of VFAs accumulation occurs along with sequential rise in proton concentration (Swetzenbaum et al., 1990). pH drops as a result of consecutive increase of proton concentration that has an inhibitory effect on methanogenesis. Eventually, it causes significant lower biogas yield and reactor failure afterwards. Cord-Ruwisch et al. (1996) reported digester failure due to increase in H_2 concentration originated from organic shock loading.

2.5.2 Hydraulic overloading

It has been reported that vigorous acetic and propionic acid accumulation takes place during hydraulic overloading (Mosey and Fernandez, 1989). Kennedy and Van den Berg (1982b) observed increase in acetic and propionic acid concentration by 8-10 folds higher than the normal level caused by hydraulic overloading to 0.78 d (HRT 1.3 d) treating chemical waste using anaerobic fixed film reactor. As a result, overloading of 60-70% compared to the normal level of 11 g COD/L/d took place due to sudden reduction of HRT. While imposing a hydraulic shock loading due to reduction in HRT from 10 to 7 d, Conivas-Diaz and Howell (1988) reported propionic acid predomination during treatment of cheese-whey wastewater using anaerobic fixed film reactor.

2.6 ANAEROBIC CO-DIGESTION

Co-digestion is the instantaneous digestion of an analogous blend of two or more substrates (Wu, 2007). Conventionally, AD was a solitary substrate, solitary resolution technology. Freshly, it has been recognized as reliable technology, while the combination of substrates increased treatment efficiency with high biogas yield.

The most usual cases is while a core quantity of a basic substrate such as manure or sewage sludge is blended and digested combined with less volume of a solitary, or a multiplicity of supplementary one (Braun, 2002). Application of co-substrates generally accelerates biogas generation from AD process because of positive synergisms built in system and feed of lost nutrients through co-substrates (Mata-Alvarez et al., 2000).

2.6.1 Advantages and limitations of co-digestion

Numerous potential environmental, technical and financial benefits and limits are projected in Table 2.5. Table 2.6 illustrates methane yield using different animal manures.

Table 2.5 Benefits and Boundaries of co-digestion treatment (Braun, 2002)

Advantages	**Limitations**
Better nutrient balance and digestion	Augmented reactor effluent COD
Equalization of particulate, floating, settling, acidifying, etc. wastes, via dilution by manure or sewage sludge	Supplementary pre-treatment necessities
Supplementary biogas generation	Improved mixing necessities
Potential gate fees for waste treatment	Wastewater treatment prerequisite
Supplementary fertilizer reclamation	High utilization degree required
Renewable biomass ("Energy crops") disposable for digestion in agriculture	Decreasing availability and rates

Table 2.6 Methane yields of manures (Moller et al., 2004a, b, and Hashimoto et al., 1981)

Feedstock	**Methane yield m^3 per kg volatile solids**	**Reference**
Manure		
Pig	0.36	Moller et al. (2004a,b)
Sow	0.28	Moller et al. (2004a,b)
Dairy cattle	0.15	Hashimoto et al. (1981)
Beef cattle	0.33	Hashimoto et al. (1981)

It is obvious that digestion strategy is capable enough to enhance methane production compared to conventional methods. In this point, anaerobic co-digestion of petrochemical waste water with dairy and cattle manure was carried out.

2.6.2 Supplementation of NH_4HCO_3

In order to facilitate co-digestion efficiency ensuring proper nutrient balance and buffering capacity supplementation of NH_4HCO_3 was employed. It has been studied that insufficient buffering capacity and distraction of bacterial population equilibrium between non-methanogen and methanogen to transform carbonaceous organic to CH_4, were identified to be the key reason of operational failure. To maintain VFA concentration during process, alkalinity should be controlled by recirculation of treated slurry (Borja and Bank, 1996 and Najafpour et al., 2006) or supplementation of lime and bicarbonate salt (Gerardi et al., 2003). As this process has been shown to be a proficient alternative both to pollution control and to produce CH_4 as the bioenergy, hindrances while operation should be mend.

2.7 IMPORTANT CALCULATION METHODS FOR ANAEROBIC TREATMENT PROCESS

2.7.1 The F/M Ratio

F/M ratio is the main factor dominating AD. Contaminant concentration and microbial mass is accommodated by F/M. Moreover, F/M is the mass of pollutant applied to a unit mass of microbial mass per unit time (e.g. g COD/ g VSS day).

Lower F/M ratio will cause a superior proportion of substrate being transformed to gas. Performance of a reactor may be enhanced by dropping F/M ratio and rising biomass loading.

The F/M ratio varies between 0.1-0.6 where low ratio contributes in high biogas yield.

$$\mathrm{F/M} = \frac{\mathrm{CODin}}{\mathrm{HRT \times VSS}} \tag{2.17}$$

2.7.2 Hydraulic Retention Time

HRT can be defined as the mean time that a fluid resides in a reactor. In other words, HRT is the time consumed by a liquid entering from inlet to the outlet until all soluble matter could be degraded. Followed by the F/M ratio, the hydraulic retention time (HRT) can be calculated as,

$$HRT = \frac{CODin}{OLR} \tag{2.18}$$

2.7.3 Flow Rate

The ratio of working volume of reactor and HRT can be defined as flow rate. The lower will be the diameter of tube hose; less will be the flow rate of the influent stream and vice versa. It can be calculated by the equation below,

$$Q = \frac{Vw}{HRT} \tag{2.19}$$

2.8 CONCLUSIONS

The definition and functionality of chemical and biological coupled treatment technology and review of other relevant research studies are covered. The prevailing problems of reactor operation have been reviewed. Other pretreatment methods, high rate anaerobic reactors, advantages and drawbacks of aerobic and anaerobic digestion techniques replication, and anaerobic co-digestion technology are reviewed.

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

This section explains the research framework. Materials, experimental set up, inoculum, analytical methods have been demonstrated clearly. The details of experimental outcome have been discussed further in the relevant chapters.

3.2 RESEARCH FRAMEWORK

In the proposed system, combination of chemical and biological treatment technology focusing on hydrolysis and methanogenesis was tried. With a view to achieve the research objectives, H_2O_2 were selected as pretreatment tool and continuous stirred tank reactor (CSTR) was employed as the biological treatment tool. However, in each case batch treatment was performed strictly. On the basis of batch treatment results, reactor operation was carried out. Batch treatment was performed with trial and error methods. Statistical analysis was also worked out with a view to check the validity of the results. Figure 3.1 elaborates the research framework to provide a crystal clear view on the experimental strategy.

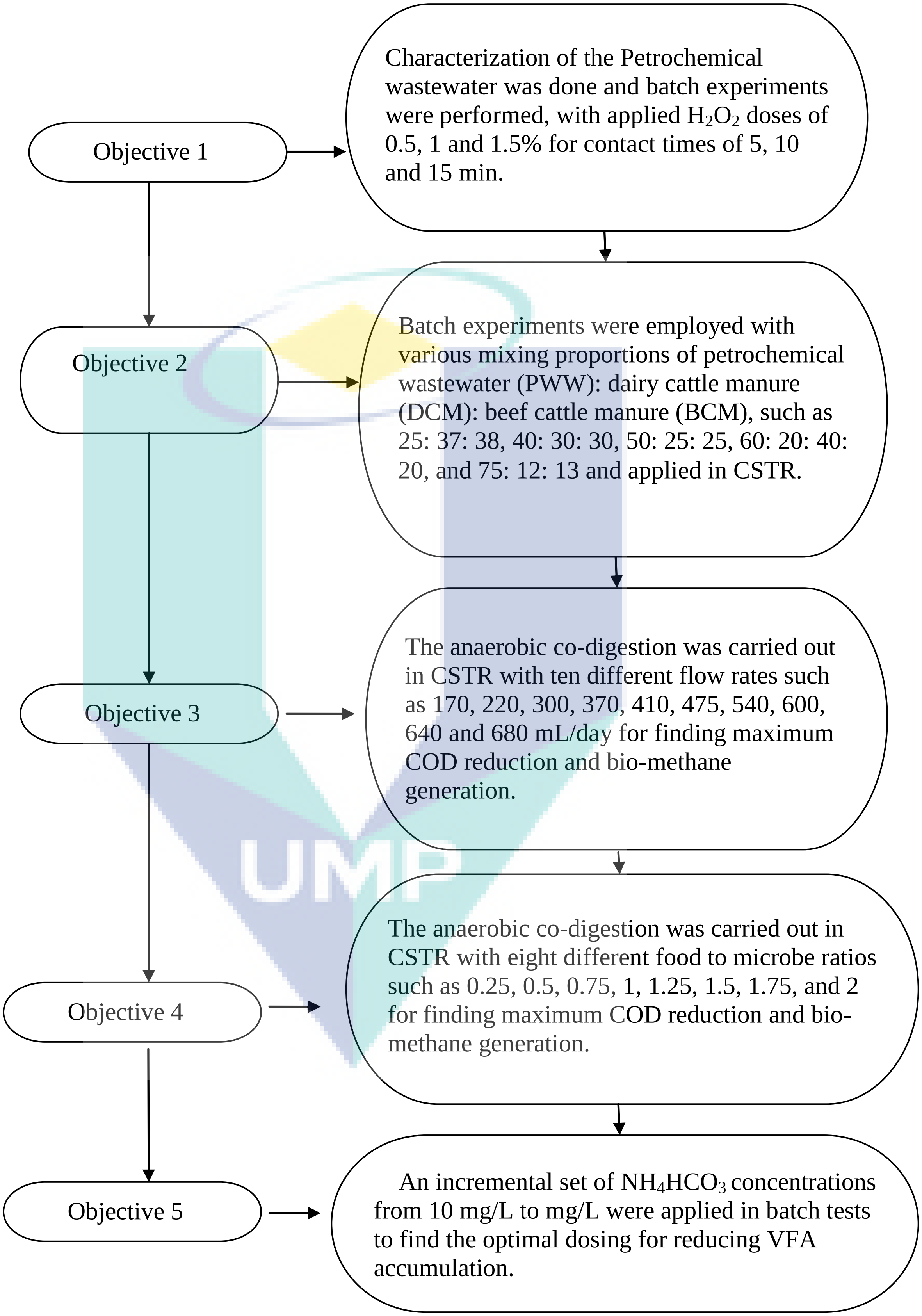

Figure 3.1: Structure of experimental plan

3.3 SAMPLE COLLECTION

A 100-L sample of petrochemical waste water (PWW) was accumulated in containers from discharge receiving stream of the Petronas Penapisan (Terengganu) Sdn Bhd (petroleum refinery) at Terengganu, Malaysia. However, hydrocarbon degrading bacterial strain *pseudomonas aeruginosa* ATCC 27853 was purchased from BioSynTech Malaysia Group Sdn Bhd as it provides the maximum degradation efficiency. The main petrochemical product of this refinery is naphtha. The capacity of this refinery has been reported as 2.4 million (metric ton per annum) mtpa. However, naphtha, usually denotes to numerous combustible liquid mixes of hydrocarbon, that is a constituent of natural gas condensate or a refinement outcome from petroleum, coal tar or peat boiling in a definite limit and comprising specific hydrocarbons. It's an extensive covering among the lightest and most volatile portions of the liquid hydrocarbons in petroleum (Rune et al., 2004). Naphtha is a colorless to reddish-brown volatile aromatic liquid, very similar to gasoline (Rune et al., 2004). As observed the company at this moment is adopting chemical treatment which can only capable to remove COD. But the treatment technology is not that effective to provide any energy as feedback. Even though, there exists a potentiality to produce significant amount of energy using anaerobic digestion process.

3.4 SAMPLE PRESERVATION

Approximately 100 kg partially digested beef cattle manure (BCM) and dairy cattle manure (DCM) was collected duly from ejection of average-sized farm in Gambang, Malaysia. The PWW, BCM and DCM were placed in compact frost containers and transferred to preservation cold room. The temperature was maintained at 4°C during preservation. Effluent pH was maintained around 6.5 by addition of 6N NaOH solution. Alkalinity was maintained around 1500–1700 mg/L $CaCO_3$ with

$NaHCO_3$. Additional nutrients like nitrogen (NH_4Cl) and phosphorous (KH_2PO4) were supplemented to maintain COD: N: P ratio at 250:5:1.

3.5 PREPARATION OF SAMPLES

3.5.1 Petrochemical wastewater (PWW)

Meanwhile for the PWW sample, it was subjected to the simple screening to eliminate coarse materials. Then it was further screened over a filter media, which was comprised of trivial stones having average dimension of 0.6 cm. The screened deposit was filtered over another bed comprised of combination of trivial stones and sand (average dia 300–600 µm) 1:2. The remainder was then exposed to surface filtration. This filtration activity was done by using a Whatman No. 41 filter paper (20–25 µm) and eventually a Whatman No. 40 filter paper (8 µm) under vacuum. Finally, the raw and filtered PWW was subjected to pH, COD, BOD_5, TS, SS, TN, TP determination.

3.5.2 Beef cattle manure (BCM) and Dairy cattle manure (DCM)

The dilution of BCM & DCM in water at proportion of 1:25 were made and filtered through sieve (20µm) to remove debris. However, thermal pretreatment of manure is effective at increasing methane production by 20% and reducing fibrous particle sizes (Angelidaki et al., 2000), which are recognized as thermal hydrolysis. Hence, the solid portion of blended manure was heated to 100–140 °C before AD, to improve methane yield and volatile solids degradation according to the procedure of Mladenovska et al. (2006).

3.5.3 Combined Activated Sludge Biomass

Combined activated sludge biomass (ASB) formation was executed according to the procedure of (Ahmad et al., 2010). For ASB cultivation, a mixed medium was employed as an energy resource: 3.0 g/L $K_2HPO_{4,}$ 0.1 g/L $MgSO_4$ .7 H_2O, 0.3 g/L $CaCl_{2.}$

2 H_2O, 0.01 g/L $FeSO_4$. 7 H_2O. Initially, pH of that medium was found 4.5. Thereafter, 150 mL PWW was added in to a 250 mL Erlenmeyer bottle having 100 mL of energy medium and was pre-cultured with aeration at 30 ˚C and 120 rpm in incubator for three weeks. Subsequently, pH at 6.5 was maintained using 1N H_2SO_4 and 1 N NaOH throughout incubation period. The culture was lastly planted to agar medium and ameliorated. The microbial medium was established, after 20 successive cultures. Eventually, the medium was cultured in 500 mL Erlenmeyer bottle comprising 200 mL of medium and PWW at 30 ˚C and 125 rpm in incubator for five days. The ultimate microbial medium was employed as combined activated sludge biomass for biological degradation of waste. The same procedure was followed in the case of BCM and DCM.

3.6 DESIGN AND FABRICATION OF CSTR

The conventional CSTR design is not capable enough to maintain the pH and temperature in an appropriate way. pH, temperature sensor and digital pressure display were installed to obtain a good control over the system. Figure 3.2 shows a computer aided design of CSTR. Figure 3.3 shows a CSTR reactor used in this study. The reactor was fabricated in university workshop. The dimension of CSTR reactor used in this study was measured to determine the reactor’s total and working volume. The reactor was made of cylindrical Global configuration is a system resource where all the static data, class, etc. The main digester is constructed of glass and stainless steel. It has been strictly sealed with steel plate capping in conjunction with six nuts.

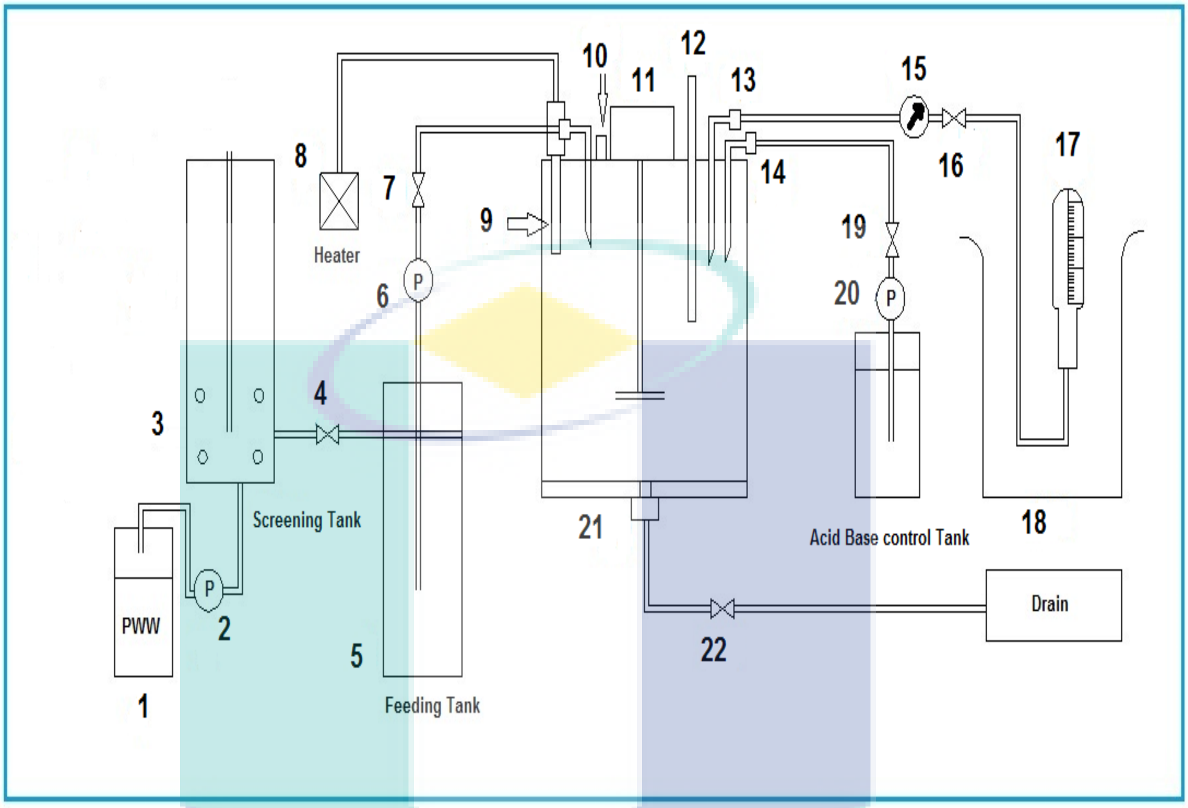

Figure 3.2 Experimental setup of CSTR. 1-Raw PWW dosing zone; 4,7,16,19,22- control valve; 3-screening tank, 5- feeding tank; 2,6,20-peristalic pump;8-heater; 9- temperature sensor; 10-pressure controller; 11- Stirrer Motor with stirrer;12-pH sensor; 13-Gas collection wire; 14-acid base control wire; 15-biogas flow meter; 17-biogas collection tank; 21-Process reactor (capacity 4.5 L, working volume 2.7 L); 18-water displacement system.

Figure 3.3 Photograph of experimental set up.

The stirrer motor is installed over the plate. It can also be controlled with a speed control device. It has a range from 100-260 rpm. In order to maintain the temperature, heater has also been installed in the system. Feeding tank to serve feeding has also been added. Biogas can be collected through a biogas collection tank. The design specification is provided in Table 3.1.

Table 3.1 Design specifications of CSTR

Specification	Measurement	Units	Remarks
Main digester			
Internal diameter, D_{in}	14	cm	
Height, h	29	cm	Total height of the reactor
Total volume, V_r	4.5	L	
Working volume, V_w	2.7	L	
Cross-sectional area, A	153.86	cm^2	
Feeding tank			
Internal diameter, D_{in}	15	cm	
Height, h	20	cm	Total height of the feeding tank
Total volume, V_r	3.53	L	
Working volume, V_w	2.12	L	
Cross-sectional area, A	176.63	cm^2	
pH controller			
pH meter	1	-	BL 931700
Temperature controller			
Digital Temperature device	1	˚ C	JCS Shinka
Pressure controller			
Digital Pressure device	1	mm H_{20}/d	JCS Shinka
Stirrer speed controller			
Speed control unit	1	rpm	SPG Co. Ltd
Feed pump			
AC adapter input		V	230V, Deng Yuan
AC adapter output		V	13.5V, Deng Yuan

3.7 PRETREATMENT (OXIDATION BY H_2O_2)

Four wastewater samples of equal volume (150 mL) were placed in conical flasks. Thereafter, each of the three volumes of wastewater was treated with 50 mL standard volume of 30% H_2O_2 solution and 1.0 mM Fe^{3+}. To find the optimal dose of H_2O_2 solution for better degradation, the percentage of H_2O_2 added was gradually increased (i.e. 0.5% to 1% and 1.5%). The liquid content of effluent with H_2O_2 was agitated for 30 min using a mechanical device. The optimal H_2O_2 concentration (1%) was predetermined by a preliminary study that aimed to achieve elevated biodegradability through biological oxygen demand BOD_5: COD ratio. Application of 2.5 hours oxidation by H_2O_2 achieved approximately 49% enhancement in biodegradability from 0.53±0.12 to 0.79±0.06 accompanied by 35% COD reduction. Appendix G and H show the batch test procedure.

3.8 CATALASE ACTIVITY, OH^- MEASUREMENTS

Catalase can be defined as an enzyme that disintegrates H_2O_2 into oxygen and water. Chance et al., 1979 stated that H_2O_2 is a normal metabolic in living cells. As excess of H_2O_2 may oxidize cellular components, so removal of H_2O_2 is necessary (Gaetani et al., 1989). Following the Worthington enzyme manual the catalase (units/ mg) activity test was done in the lab with the help of UV Spectrophotometer of Perkin Elmer Lambda 25. The maximum wave length 245 nm was fixed through scan. Hence, spectrophotometer was adjusted at 245 nm and 25 °C. Reagent grade water of 1.9 mL and 0.059 M of 1 mL H_2O_2 was pipette in to cuvette. Incubation was performed in spectrophotometer for 4-5 minutes to obtain temperature equilibration and to create blank rate. Addition of 0.1 mL of diluted enzyme made reduction in absorbance at 245 nm for 2-3 min. Alkalinity OH- was measured through titration method.

3.9 BATCH TEST TO FIND THE OPTIMAL NH_4HCO_3 DOSING

Immersing a set of air sealed digesters (1 L) in a water bath; the effect of NH_4HCO_3 on the anaerobic digestion of PWW was investigated. The operating temperature was adjusted at 37˚ C. In order to monitor biogas generation, the digesters were linked to biogas measuring device. All digesters were seeded with 300 mL of stabilized sludge and 150 mL of PWW with COD of 3000 mg/L, before testing by batch operation. The reason behind it was to assume non-critical organic loading at 0.5 kg m^{-3} so that the shock loading to seed substrate could be avoided. An incremental set of concentrations from 10 mg/L to 40 mg/L were prepared in duplicates of five containers via dosing of NH_4HCO_3. The supplementation dosing from 10 mg/L to 40 mg/L was preferred to find the optimal value. To ensure sufficient mixing and to assist the yield of biogas, all digesters were mildly stirred every 10 min. The optimum dosing for NH_4HCO_3 was calculated depending on the cumulative biogas yield. An assumption might be made that accelerated biogas yield would generate within 3 h of batch process for similar substrate (Redzwan and Banks, 2004). Nonetheless, the toxicity of NH_4HCO_3 especially to the methanogen in system could be indicated in contrast of the maximum biogas yield (Sawayama et al., 2004; Redzwan and Banks, 2007). Even so, the biogas generation in this work was assumed to be too negligible for analysis by gas analyzer. Liquid displacement method was applied to measure gas generation. For the calculation of % increase in biogas yield the following formula was employed:

$$\%\ \text{increase in biogas yield} = (A - B) / B \times 100 \qquad (3.1)$$

Where, A = Biogas yield at dosing 10 mg/L
B = Biogas yield at dosing 0 mg/L (control)

3.10 BATCH TEST STUDIES

Plastic bottles with a capacity of 1.5 L were selected as digesters. This was a modified form of the compact system digester that digests small volumes of manure to generate biogas. A thermometer was placed in each digester to measure the temperature. The gas pressure was measured by a U tube manometer and pH 6.5 was maintained by a digital pH meter using 1 N H_2SO_4 and 1 N NaOH solution. The mean of three readings was calculated. The maximum methane yield was found at PWW: BCM: DCM (50:25:25) ratios.

3.11 FERMENTER, INOCULUM MATURATION AND REACTOR OPERATION

Solitary-phase anaerobic co-digestion of PWW, BCM and DCM was performed in a 4.5-L (total volume) (working volume 2.7 L) (Figure 3.3) CSTR. It was furnished with digital temperature, pH and pressure control device. It had a stirrer with two propellers to ensure uniform mixing, and rotating speed could be maintained between 0 to 1200 rpm. An electronic heater was installed with the main digester to provide desired conditional temperature up to 75 °C indispensable for AD. The pH of the system was maintained through a dosing pump installed to acid and base feeding tanks. A water displacement system was used to measure biogas volume.

The CSTR was started up using partially digested BCM: DCM at proportion of 1:1 as sole substrate. Reactors were run at 37 and 55 °C for 30 days during inoculum development. Each reactor was fed with 675 mL of ready mix of the same every 5 days until 2.7 L of its working volume was achieved. Mixing was generated by direct electronic motors joined to stainless steel blades functioning at 60 rpm for 6 min/h. Meanwhile, the anaerobic bacteria consumed organic compounds in the sludge as a substrate and formed anaerobic environments suitable for the development of firm anaerobes. On alternate days, the slurry was slowly replaced by PWW: BCM: DCM (50:25:25) at pH 6.5 as substrate. It was continued up to HRT of 20 days for around 55 days. Afterwards, PWW was fed to the reactor continually with BCM and DCM , beginning with HRT of 20 days and gradually altering to the anticipated HRT, with a gradual rise in organic loading, which allowed steady-state conditions eventually to be accomplished. During these phases, 135 mL/day was taken out from upper layer of the mixed wastewaters prior to mixing to avoid biomass loss, and served with 135 mL PWW supernatant. Under these conditions, the reactor achieved a constant COD reduction at fixed HRT and OLR, which were defined as steady-state conditions. At 37 °C, 98±0.5% COD reduction was achieved at 10 days HRT, which confirmed adaptation of methanogens for pH and contaminated constituents of PWW. Reactors were run under both mesophilic (37 °C) and thermophilic (55 °C) conditions on an uninterrupted basis at anticipated holding period (HRT) for 65 days while attaining steady-state. AD of control PWW was operated simultaneously. The properties of fed wastewater and effluent were analyzed thrice weekly, excluding pH that was examined daily. The outcomes of the analysis of each stage at steady state were taken in to account for assessing the consequence of co-digestion on biodegradation, biogas generation productivity and additionally system stability.

3.12 OPERATIONAL DESIGN FOR CO-DIGESTION OF PETROCHEMICAL WASTEWATER AND ACTIVATED MANURE

The typical operating parameters used in full-scale manufacturing plants may be adapted for design-scale AD plants. The PWW and AM were subjected to an automated treatment to decrease particle size. Afterward, 96 % of the PWW grains had a size from 2 to 4 mm, and the remaining 4 % surpassed 4 mm; 100 % of AM particles had a size below 1 cm. These features are noted in the technical literature as essential parameters in AD (Sun et al., 2008; Kaparaju et al., 2000). The smaller the grain size, the greater the efficacy of the system because a decline in grain size suggests a rise in the surface area on which microbes can act. The dense remainder was homogenized, and then blended with other wastes according to the above-noted ratios. For co-digestion, waste samples were mixed from the raw by-products (PWW and AM) according to the selected proportions.

Wastes from petrochemical plants lack the appropriate microbes to activate a bio-degradation process. Therefore, an acclimatized inoculum is essential. AD is used for tertiary treatment to further treat the effluent from primary and secondary units. After transport to the laboratory, the inoculum was transferred into the CSTR immediately. Once entirely packed with the inoculum, the CSTR was checked for leaks to guarantee an oxygen-free degradation process, and the flow of influents was measured to analyse feeding the next day. Triplicate experiments were carried out with raw wastes and combinations thereof, using several flow rates. Every experiment was performed for an adequate span of time to confirm reliable results in terms of the degree of digestion and CH_4 production. The optimal period for every trial was computed based on HRT, which is the length of time that waste is in contact with microbes. It was determined as the ratio between the CSTR volume and the volume of waste added daily. For instance, given an inflow of 250 mL PWW/ day, the HRT is 8 d; therefore, on this occasion the test was performed for a minimum of 16 days. The initial HRT represents the acclimatization period before reaching steady state. The AD process reached equilibrium when waste COD remained steady at a minimum value and the daily amount of methane generated was steady and at a maximum.

In every experiment, the waste flow was steadily increased so that the microbes could adapt to the new environment. It was increased up to the level at which surplus organic loading occurred. After completing all designed experiments, the optimal flow of each combination of wastes was determined based on maximum COD removal and methane generation. Ultimately, the digested effluents were collected after AD treatment of PWW and AM to analyse their composition.

3.13 OPTIMAL FLOW RATE

The sample of PWW was co-digested anaerobically with AM in a 4.5 L glass CSTR, which was connected to digital temperature and pH control meter (Figure 3.3). The CSTR could provide homogeneous mixing with a stirrer of two paddles. The speed of mixing could be controlled using an electronic regulator between 0 to 1200 rpm. An electronic heater was connected to the main digester to supply heat up to 75 °C to maintain the suitable condition for microbial growth. pH variations were maintained by two dosing pumps connected to acid and base buffer solution tank. A flow meter was installed to the CSTR to maintain the suitable wastewater flow rate. A water displacement method was used to measure biogas.

The CSTR was initially run with AM as inoculation substrate. The CSTR was fed with 650 mL of seed sludge each five days until it reached 2.7 L of its working capacity. Uniform mixing was provided with two electric motors connected to the stirrer paddles operating at 60 rpm for 6 min/h. In the meantime, using the organics of the seeds, facultative anaerobes developed an oxygen free condition suitable for their development. On the next days, the seeds were gradually substituted by PWW: AM (50:50) at pH 6.8 as feed wastewater, at 15 d HRT for nearly 54 d. Later, PWW was filled regularly to the digester, starting with 15 d HRT and steadily changing to the desired HRT, by a slow addition of organic loadings (OLR), which allowed for steady-state to be reached. At this phase, 130 mL/day was taken out from upper layer before mixing to prevent biomass loss, and replaced with 130 mL PWW supernatant. Maintaining these states, the CSTR realized a consistent COD removal at constant loadings and retention times, which indicated the steady-state period. Ten trials with different flow rates were performed with similar preliminary conditions of the reactor. During every trial, partial outcomes and the development followed by the influent concentration were observed. The results from each analysis of distinct trial at steady period were responsible for observing the influence of flow rates on biogas conversion and sustainability.

3.14 FINDING OPTIMAL F/M RATIO

The CSTR operation was separated into two experimental trials: trials I and II. The design strategy for each trial is listed in Table 3.2. Both trials I and II were performed in 4.5 L glass CSTR (liquid volume 2.7 L) (Figure 3.3). The F/M proportion was gradually increased from 0.25 to 2.0 by adding the organic loading from 6.31 to 27.14 g VS/L at a fixed inoculum loading rate of 4.0 g VS/L. The F/M proportion was determined based on the primary VS of the feed sample and inoculums (Kafle and Kim, 2013a). After the addition of the necessary volume of co-substrate and inoculum, each CSTR was fed with tap water to adjust the required volume. To assure anaerobic conditions, each CSTR was tested for airtightness and flushed with 100% pure nitrogen nearly for three minutes (Chandra et al., 2012). The mesophilic CSTR was run at 37 ºC, and thermophilic CSTR was run at 55 ºC in a heat controlled glass vessel. Both trial I and II were performed in triplicate, and the outcomes were listed as an average. Mixing was generated by direct electronic motors joined to stainless steel blades functioning at 60 rpm for 6 min/h.

Table 3.2 Operational design strategy for the run of CSTR

	Temperature	**Organic loadings (gVS/L)**	**F/M ratio**	**No. of repetitions**
Trial 1	37	6.31	0.25	3
		7.5	0.5	3
		8.99	0.75	3
		12.72	1	3
		15.73	1.25	3
		20.15	1.5	3
		25.17	1.75	3
		27.14	2	3
Trial 2	55	6.31	0.25	3
		7.5	0.5	3
		8.99	0.75	3
		12.72	1	3
		15.73	1.25	3
		20.15	1.5	3
		25.17	1.75	3
		27.14	2	3

3.15 ANALYTICAL METHODS

The COD, BOD, TOC, TN, TKN, TP, VFA, total solids, total suspended solids and volatile suspended solids, pH, alkalinity were measured by the Standard Methods of waste and wastewaters (APHA, 2012). A List of apparatus used to determine parameters are shown in Table 3.3.

Table 3.3 List of apparatus used to determine parameters

Parameter	**Apparatus**
COD	HACH apparatus
BOD	HACH apparatus
BDOC	HACH apparatus
TOC	HACH apparatus
TN	HACH program 350N
TKN	Digesdahl digestion apparatus
TP	HACH apparatus
TS	Centrifuge method
TSS	HACH apparatus
VSS	HACH apparatus
VS	HACH apparatus
pH	HACH pH meter (Sension 1)
Alkalinity	Titration apparatus
C and N	Thermo Finnigan Flash EA 1112 elemental analyzer
Biogas and Methane	Shimadzu Class-GC 14B gas chromatography apparatus
VFA	Shimadzu Class-GC 14B gas chromatography apparatus

3.16 STATISTICAL ANALYSIS

The data was analyzed for three replicates by using Microsoft Excel 2010. All necessary statistical data was derived in this software. The mean, standard deviation and standard error results were calculated from replicates by Origin pro 8.6 and applied to each Figure and table values.

3.17 CONCLUSION

The experimental design and analytical methods are discussed in this chapter. Sample collection, characterization, preparation also has been demonstrated. The details of reactor design specifications have been covered. The activated sludge process, inoculum preparation and characterization have been thoroughly described. All these discussion makes a better understanding on research methodology.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 INTRODUCTION

This episode describes implementation, experimental outcomes and discussion of petrochemical wastewater (PWW) treatment using CSTR reactor. In this part, results are described in terms of effect of mixing ratio of PWW, DCM and BCM, effect of temperature on VFA and pH, effect of organic loading rate (OLR), hydraulic retention time (HRT) and food to micro-organism ratio (F/M) on reactor performance at mesophilic and thermophilic conditions, effect of pH and VFA: alkalinity ratio, VFA accumulation, C/ N ratio and methane generation potential are described. Purpose of this implementation is to illustrate the usefulness and feasibility of the chemical biological coupled technology described in the previous chapter. Finally, the results have been compared with other process.

4.2 CHARACTERIZATION OF PETROCHEMICAL WASTEWATER

The raw PWW was characterized in terms of the parameters pH, BOD, COD, TS, TN etc. The PWW characterization has been summarized in Table 4.1.

Table 4.1 Composition and Characteristics of PWW

Parameters	**PWW**
pH	6.2±0.2
BOD	7.85±0.13
COD	15.2±0.5
TOC	4.98±0.05
Total solids	0.32±0.05
Acetic acid	1.5±0.03
Phenol	0.36±0.2
Total Nitrogen	0.048±0.005
Total Phosphate	0.106±0.004

Except pH all parameters in gL^{-1}

Values are the mean + S.D. of the 3 determinations

Table 4.2 shows the filtered values obtained for each parameter determined for PWW.

Table 4.2 Composition and Characteristics of filtered PWW

Parameters	**Raw PWW**	**Filtered PWW**
pH	6.2±0.2	6.12±0.2
BOD	7.85±0.13	7.65±0.13
COD	15.2±0.5	15±0.5
TOC	4.98±0.05	4.95±0.05
Total solids	0.32±0.05	0.3±0.05
Acetic acid	1.5±0.03	1.02±0.03
Phenol	0.36±0.2	0.35±0.2
Total Nitrogen	0.048±0.005	0.045±0.005
Total Phosphate	0.106±0.004	0.102±0.004

Except pH all parameters in gL^{-1}

Values are the mean + S.D. of the 3 determinations

4.3 H_2O_2 ON PHYSICOCHEMICAL CHARACTERISTICS OF PETROCHEMICAL WASTEWATER

Table 4.3 represents biological fermentation of PWW with different applied H_2O_2 doses under semi-batch test operation. The implemented H_2O_2 doses ranged between 0.5% -1.5% and whereas oxidation continued from 2 to 30 min.

The per-oxidation efficiency promoting effluent class was firmly depended on applied H_2O_2 dose. In case of current study 5 min OHP period was noticed most substantial for extreme removal of contaminants, longer OHP period did not suggestively influence the general properties of the waste. Volk et al. (1996), and Kim et al. (2010) reported 6.5 mg/L ozone & H_2O_2 doses (ratio 0.35 mg H_2O_2/ mg O_3) at contact time of 10 min was found to be effective for higher removal of pollutants in waste. Similarly from Table 4.3 31% and 28% COD and TOC removal was realized in case of PWW, at 1% H_2O_2 dose. Mater et al. (2007) reported 28% TOC removal at1% H_2O_2 and 1mM $Fe3^+$. Results showed that a H_2O_2 dose of 1% was noticed optimal, as higher H_2O_2 doses were not so effective in pollutants removal.

Table 4.3 Biological fermentation of PWW with applied various doses Hydrogen peroxide

Parameters	**H_2O_2 doses**									**ASB**
	0.5%			**1%**			**1.5%**			
	5min	10min	15min	5min	10min	15min	5min	10min	15min	
pH	6.50	6.50	6.50	6.50	6.50	6.50	6.50	6.50	6.50	6.50
T OC	4059	3930	3865	3564	3430	3356	3267	3212	3202	1440
COD	12009	11708	11498	10358	10257	10156	9457	9357	9351	3002
TN	37	37	36	36	33	32	32	31	31	20
VSS	4587	4563	4546	3916	3892	3864	3790	3785	3781	1774
BOD_5/COD	0.51	0.51	0.51	0.57	0.57	0.57	0.65	0.65	0.65	0.59

ASB- Activated sludge biomass

Except pH all parameters in mgL^{-1}

Also, H_2O_2 dose higher than 1% has toxic effect on survival of microorganisms. As seen, TN removal was very low and did not exceed 29.46%. The substantial enhancement in ammonia oxidation latent in comparison with non-oxygenated controls during treatment process is the reason of low TN removal, as reported by Jennifer et al., 2010. VSS removal was significantly dependent upon H_2O_2 dose. However, OHP pathway consists of a strike by hydroxyl radicals on C-H bond of fatty acids. Aromatic rings joined to hydroxyl groups exist in fatty acids are ruptured by powerful OH radicals. This results in development of water-soluble mixtures by means of cogitation of hydrogen and inclusion of oxygen atoms in presence of ferrous and ferric ions. The consequence of this reaction yields minor aliphatic compounds, resulting from eradicating of the larger hydrocarbon chains of fatty acids which eventually bring on mineralization of the antecedent organics. Organic substances in petroleum are utilized by microorganisms as nutrients, and are transformed in to simple end products (Sumathi et al., 2004, and Martin et al., 2008).

The influence of H_2O_2 dose upon effluent properties at 5 min exposure was depicted in Figure 4.1. COD and TOC removal was 32% and 28% at 1% H_2O_2 dose.

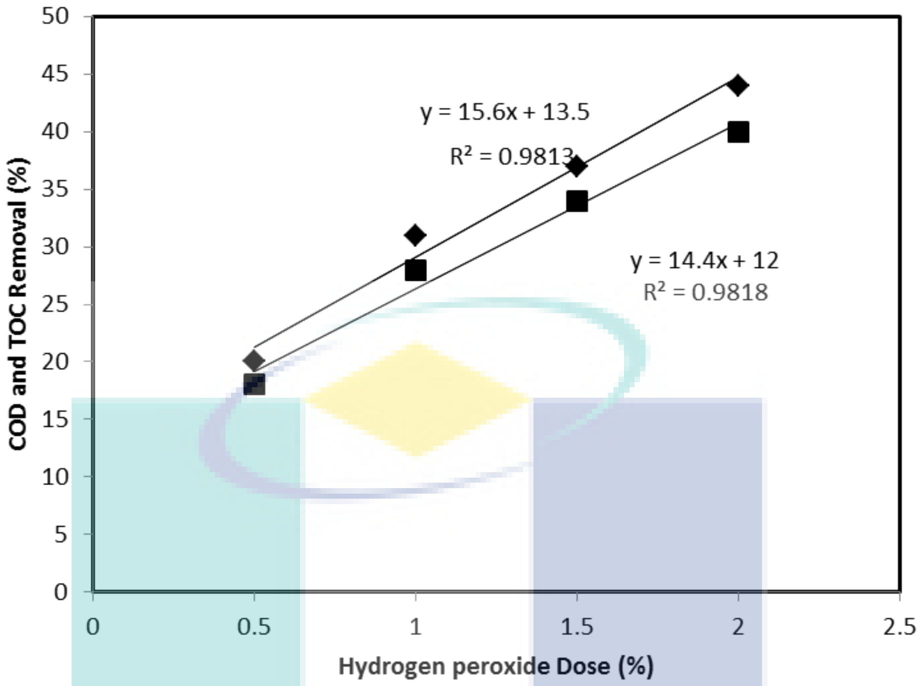

Figure 4.1: Effect of OHP on COD and TOC removal in PWW at different H_2O_2 dose (♦: COD; ■: TOC removal %)

4.4 H_2O_2 ON BDOC ACTIVATED SLUDGE & CATALASE ACTIVITY

H_2O_2 oxidation impact upon BDOC was shown in Figure 4.2. BDOC is the indicator of bacterial re-growth potential in terms of biodegradable organic matter in the wastewater (Tripathi et al., 2011). BDOC portion contains organic compounds which can be used by heterotrophic bacteria as a supply of energy and carbon. At applied dose of 1% OHP with 5 min contact time, 35% improvement was realized in case of PWW, whereas BDOC value increased from initial value 0.90- 1.08 to 1.17-1.22 mgL^{-1} for PWW.

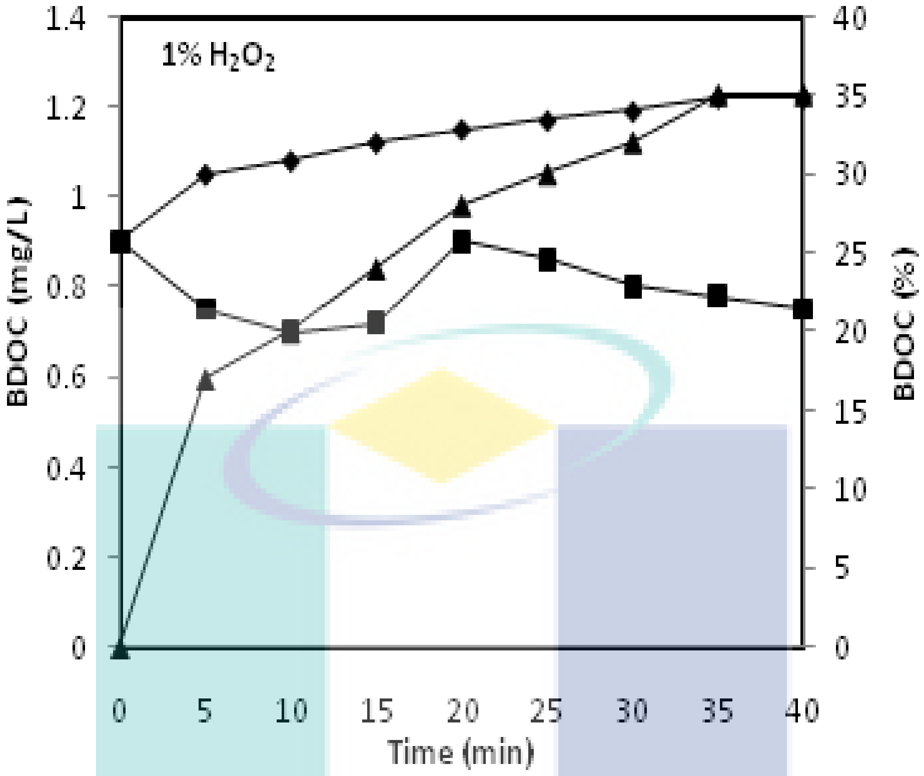

Figure 4.2 Increase in BDOC at 1% H_2O_2 dose with different exposure duration in PWW (■: BDOC initial; ♦: BDOC final; ▲: BDOC increasing %)

The reaction between H_2O_2, aromatics and unsaturated organic compounds in the waste yields formation of saturated poly-carbonaceous composites; furthermore, they acts on aliphatic acids generating OH radicals. Consequently, BDOC increases (Volk et al., 1996, and Khan et al., 2009). The reason may be due to vastly bifurcated and cyclic saturated hydrocarbons as aromatics are more resilient to biodegradation. The result also indicated that oxidation by H_2O_2 substantially increase the biodegradability of the waste; waste treatment competence might be accelerated by oxidation.

4.5 ACTIVATED SLUDGE AND CATALASE ACTIVITY

Figure 4.3a illustrates catalase activity in activated sludge (mg g^{-1}) for PWW.

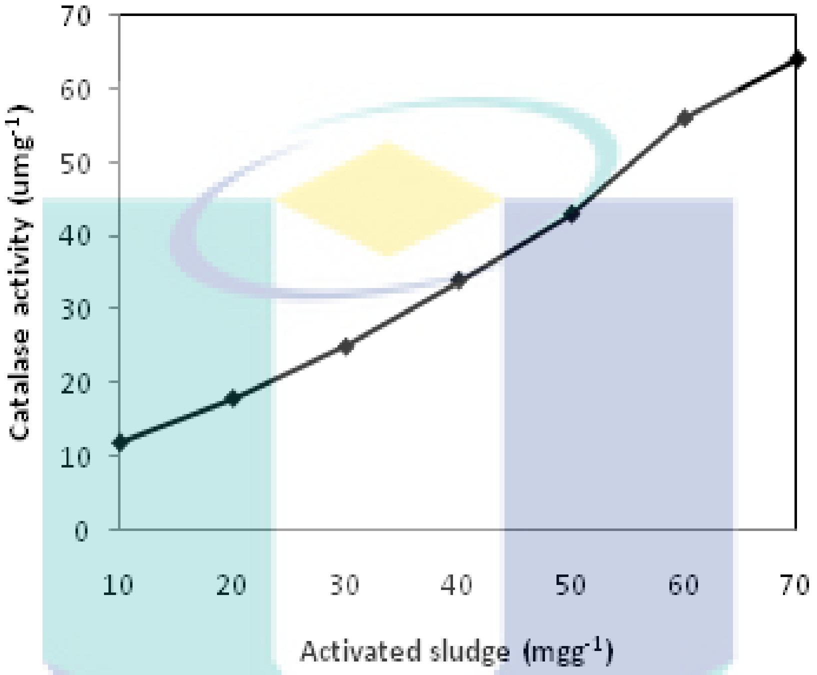

Figure 4.3a Increase in Catalase activity with respect to ASB

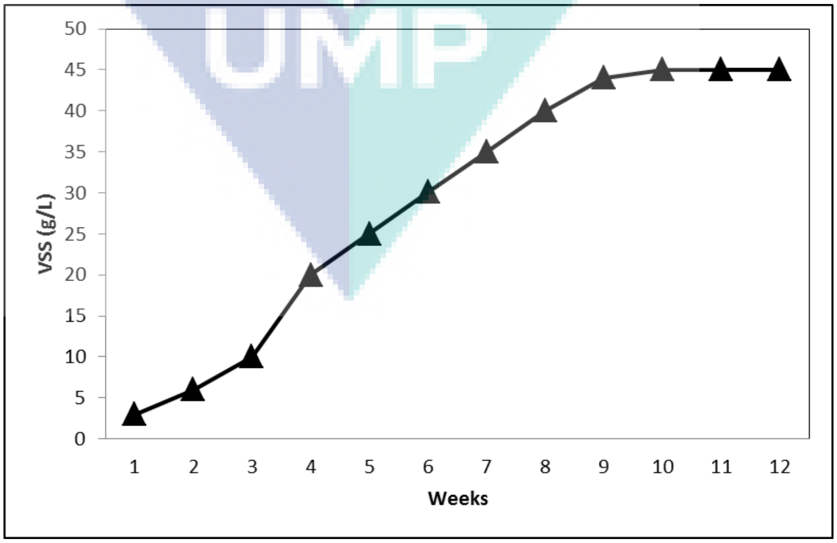

Figure 4.3b Increase in microbial activity with respect to time

Initially, when activated sludge was 10 mg g^{-1}, Catalase activity was 12 units mg^{-1} for PWW. As noted, the results indicated that catalase activity gradually increases simultaneously with the increase of activated sludge in PWW. Finally, when activated sludge was 70 mg g^{-1}, Catalase activity was found 65 units mg^{-1} for PWW. Activation of catalase requires wide range of pH (4-10) and its activity continues until pH is below 3.5 (Guwy et al., 1999). Similarly, Figure 4.3b indicates the gradual increase of the microbial activity with respect to time. As we can see looking at Figure 4.3b there is a gradual increase in the volatile suspended solid, VSS curve up to three weeks. The peak value at this time was 8 g/L. However, a sharp increase in the microbial population has been observed starting from 4th week up to the 8th week. At this stage the value of VSS was approximately 39 g/L. Subsequently, the curve becomes steady at approximately 42 g/L at the end of 10th week.

4.6 OH^- TO CATALASE ACTIVITY AND CHEMICAL TOXICITY REMOVAL

Figure 4.4 (A-C) describes the response of catalase activity on application of different H_2O_2 dose. The catalase functionality was greatly influenced by H_2O_2 dosing (Mitozo et al., 2011). Maximum catalase activity was found at 1% H_2O_2 dose. As seen, OH- toxicity was successfully scavenged by catalase activity up to1% H_2O_2 dose, whereas OH- could not completely scavenged by catalase activity at 1.5% H_2O_2 dose. As noted, OH- was yet to be scavenged by catalase activity at 1.5% H_2O_2 dose which may cause cell damage of microbes (Mitozo et al., 2011). Hence, survival of microorganisms was higher at 1% H_2O_2 dose compared to 1.5%. Mater et al. reported in 2007 that low reagent concentrations were adequate to fright the degradation method, which might be sustained by microbes.

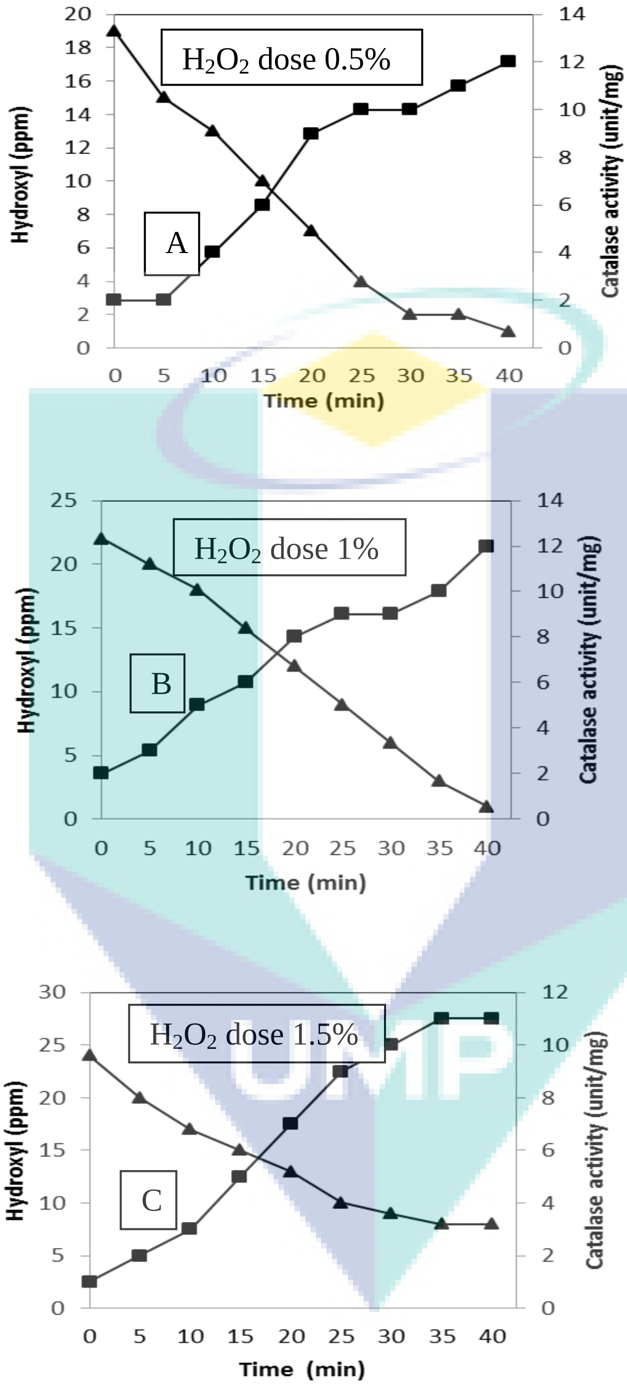

Figure 4.4 (A-C) A-Response of catalase activity (CA) at 0.5% H_2O_2 dose, **B-** Response of catalase activity (CA) at 1% H_2O_2 dose, **C-** Response of catalase activity(CA) at 1.5% H_2O_2 dose (▲: OH^- in PWW; ♦ Catalase activity in PWW)

4.7 H_2O_2 ON BIODEGRADABILITY INDEX INDICATING FATTY ACID REMOVAL

The biodegradability index (BOD_5/COD), indicating fatty acid removal in waste water, suggested being within 0.40 to 0.80 to be treated by biological process (Aboulhassan et al., 2008). Figure 4.5 describes the initial value of BOD_5/COD ratio was 0.51 in PWW and hence pretreatment was planned to be carried out by OHP. At the end of 30 min of chemical oxidation in different dosing biodegradability index (BOD_5/COD) was enhanced up to 0.8 for PWW.

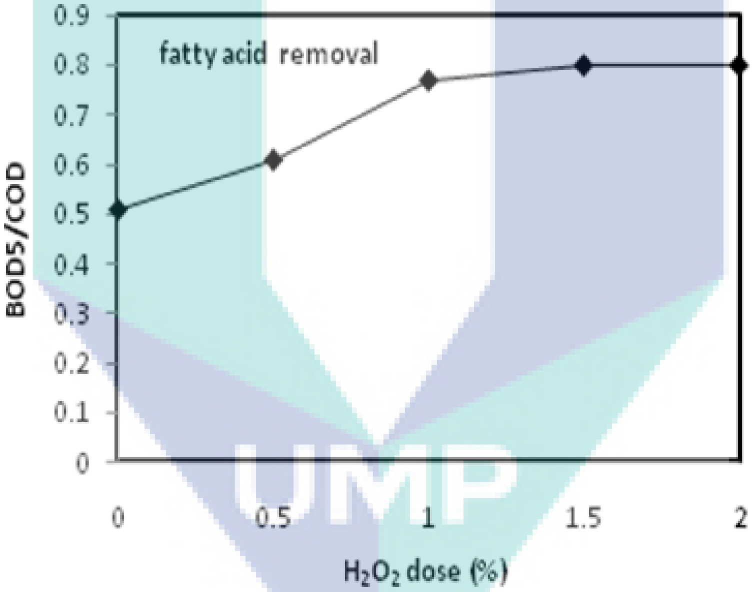

Figure 4.5: Increase in Bio-degradability index at different H_2O_2 dose (■: PWW)

The cause behind it can be explained in this way that Fenton oxidation pathway may possess the capability to attack on C-H chain of fatty acids generating hydroxyl radicals (Babu et al., 2010). Fenton process treating wastes of Palm shell transforms large molecular fatty acids in to small fatty acids like acetic acid and formic acid. It can be concluded from the result that eliminating oxidizing organics present in both POME and

PWW, the chemical oxidation by H_2O_2 removes fatty acid as reported by Mae et al., 2000.

4.8 EFFECT OF COMBINED ACTIVATED SLUDGE BIOMASS ON DEGRADATION OF PETROCHEMICAL WASTEWATER

The ASB can be defined as a mixture of microbial biomass. In order to examine the biodegradation rate of ASB in degrading pretreated waste water was tried for 5 d. Nonetheless, ASB effectively decreased TOC and COD about 71% and 80% from PWW respectively under specific operating conditions (see Figure 4.6). ASB, utilizing the organic substances as nutrients, breaks the organic matter in to simple end-product (Ballesteros et al., 2008). Mentionable that remarkable VSS removal was achieved from PWW. VSS proves biological activity occurred in wastewater. Bacteria utilizing organic substances mature and die, which eventually become part of the granules.

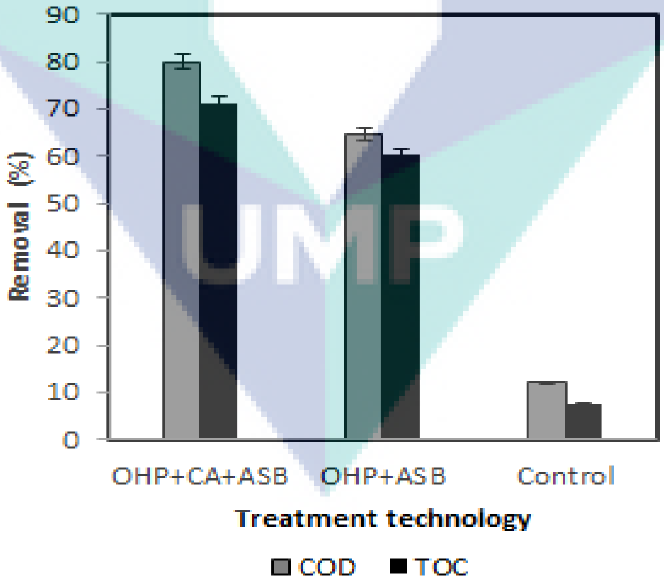

Figure 4.6 Comparison of COD and TOC removal efficiency by coupled treatment with Catalase activity (CA), coupled treatment without Catalase activity (CA) and control in PWW.

pH was maintained at 6.5 to achieve maximum biological degradation. Adeyinka in 1996 achieved 55.22% COD removal by 1% H_2O_2 dose with activated clay and sodium-ion exchange resin filtration. Babu et al. in 2010 observed 86.12% COD removal by joint electro-Fenton with biological degradation process from POME, but initial COD value was only 6712 mg/L. In another research, 49% TOC removal from petrochemical industry waste water by a solar oxidation process was obtained, where initial COD was only 2000 mgL^{-1} (Parilti, 2010).

4.9 EFFECTIVENESS OF CATALASE ACTIVITY (CA) TOWARDS ENHANCED DEGRADATION

Figure 4.6 represents the average COD and TOC removal efficiency by coupled treatment with catalase activity (OHP+ASB+CA), coupled treatment without catalase activity (OHP+ASB+CA) and control. The values were calculated based on seven days mean removal efficiency. In increasing order control< coupled treatment without catalase activity< coupled treatment with catalase activity. Control has the lowest removal efficiency whereas; coupled treatment with catalase activity was the highest compared to couple treatment without catalase activity. Hence, catalase activity has a significant influence on removal of pollutants. Scavenging H_2O_2 and protecting cells from injury instigated by reactive oxygen species are precious functions of catalases (Mitozo et al., 2011). As a result, survival of degradation capable bacteria is more in ASB, which leads to higher removal of pollutants in PWW.

4.10 NH_4HCO_3 DOSING AND DIGESTION

Figure 4.7 illustrates the effect of NH_4HCO_3 supplementation in anaerobic co-digestion process. However, the digestion performance has been evaluated in terms of cumulative biogas generation vs. time graph. It slows through termination of raw resources. While NH_4HCO_3 dosing, total cumulative biogas generation was detected to increase. More specifically, at 10 mg/L dosing and contact time ranging from 15 to 180 min, cumulative biogas generation was enhanced (Table 4.4). Subsequently, the cumulative biogas generation was detected to drop in case of 20, 30, 40 mg/L dosing applied to the process. The C/N ratio was preserved fixed within 25/1 to 30/1. Former operational breakdown that was provoked by VFA agglomeration might have happened through supplementary confines like as micronutrients (Fe, Mg, Ni, Cu, Co and P). Nonetheless, theoretically the scarcity of micronutrients might be abolished on the basis of mineral percentage. As seed sludge was collected from partially digested sewage, the content of phosphorus must be sufficient. Consequently, scarcity of phosphorus was also not being marked. For the time being, the existence of ammoniacal nitrogen as the resource macronutrient in a balanced consumed sludge is acknowledged to be at an outstanding concentration when de-nitrification is accomplished (Metcalf and Eddy, 1991). Sodium nitrate is an alternative supplementation to meet up the want of nitrogenous resource. Still, in case of its application, the discharge of NO_3^{-1} would enhance the oxidation–reduction potential (ORP) of the reactor. ORP potential of the reactor supposed to maintain above -300 mV. It was due to the cause that methanogenesis is deteriorated at lesser ORP (Gerardi, 2003). In order to adjust the buffering capacity of the substrate solution, chemical selection is a rate limiting factor.

Table 4.4 Results of NH_4HCO_3 dosing to anaerobic digestion system in terms of Cumulative biogas generation

Time (min)	**Mean cumulative biogas generation (mL) NH_4HCO_3 dosing (mg/L)**				
	0	10	20	30	40
16	15	19	18	15	15
30	18	23	24	19	19
45	23	27	27	22	23
60	27	31	30	26	27
75	31	36	34	29	30
90	36	39	38	32	33
120	40	42	41	35	37
105	43	46	43	40	41
135	46	48	46	42	43
150	49	51	49	45	46
165	51	55	51	47	48
180	52	62	53	49	49

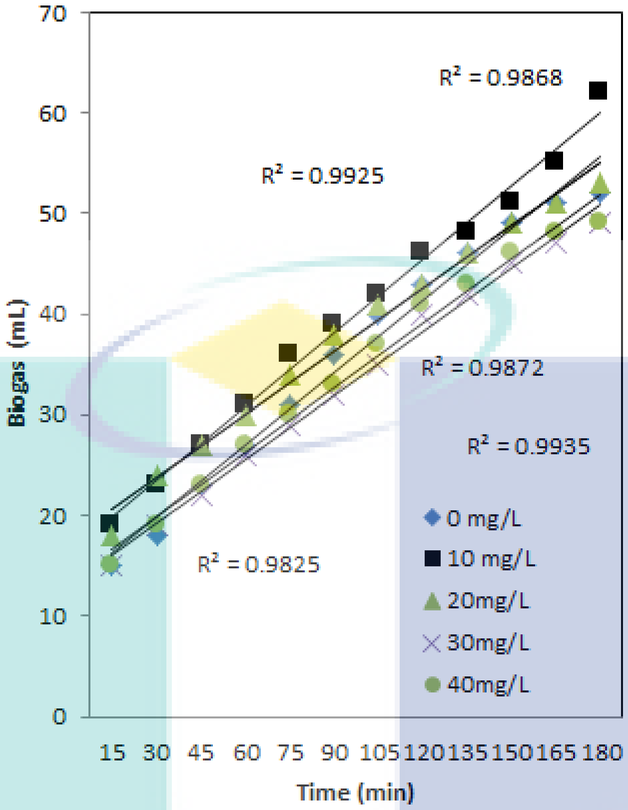

Figure 4.7 Evaluation of digestion performance in terms of cumulative biogas generation vs. time graph

However, the detailed data revealed that the maximum biogas generation took place while 10 mg/L of NH_4HCO_3 was applied. During the current work, CH_4 yield was calculated assuming similar substrate digestion. The maximum CH_4 yield from this study could be equal to 62 mL, as listed in Table 4.4. From Figure 4.7 it is obvious that the data collected during digester operation is consistent enough having regression co-efficient ranges from 0.9825- 0.9935.

The obvious effect of 10 mg/L NH_4HCO_3 dosing has been demonstrated along with contact time ranging between 15 to 180 min. From contact time vs. % increase in biogas yield curve it can be stated that the maximum enhancement in biogas yield is 27.77% at contact time of 30 min. It might be due to the fast reaction took place at that specific environmental condition. It has been studied, for the transformation of carbonaceous materials in to CH_4 during the anaerobic digestion system, sustaining methanogenesis was the key operational process. H_2 and CO_2 will be used by hydrogenotropic methanogens while acetic acid and CO_2 will be used by acetoclastic methanogens to give CH_4 as eventual outcome (Lee et al., 2012). The volatile fatty acid (VFA) accumulation is suggested to be avoided employing supplementation of strong bases and co-digestion with other wastes (Lahavand and Morgan, 2004). This strategy provides appropriate C/N ratio and strong buffering capacity to pH change. As a result methanogenesis occurs with great stability leading to enhanced CH_4 generation. It can be concluded from Table 4.4 that 10 mg/L NH_4HCO_3 dosing can provide up to 27.77% enhanced biogas yield compared to control PWW digestion.

4.11 EFFECT OF MIXING PROPORTIONS OF PETROCHEMICAL WASTEWATER, BEEF CATTLE MANURE AND DAIRY CATTLE MANURE

Table 4.5 illustrates the effect of different BCM and DCM mixing ratios on methane production. One hundred percent BCM produced more methane than 100% DCM. Hashimoto et al. (1981) has found 0.328 m^3/kg VS biogas production from BCM compared with 0.148 m^3/kg VS from DCM, due to the lower level of biodegradable material in DCM. However, the higher biogas yield from BCM might have been due to the presence of native microflora (Moller et al., 2004a and Moller et al., 2004b). Maximum methane yield was achieved by a BCM: DCM ratio of 50:50. This specific proportion was selected for reactor operation. The sovereign methane generation from these combinations might be because of an appropriate nutrient balance, which is achieved by mixing, as reported by Fulford in 1988.

Table 4.5 Effect of DCM & BCM mixing ratio on methane production

Mixing ratio	**C/N ratio**	**pH**	**Mean methane yield (mmH$_{20}$)/day**
0:100	17/1	7.5±0.20	50
25:75	20/1	7.3±0.20	110
50:50	29/1	7.2±0.20	180
75:25	33/1	7.17±0.20	56
100:0	40/1	6.99±0.20	24.5

Table 4.6 shows the mean methane potentials of different PWW, BCM and DCM mixtures. At a PWW: BCM: DCM ratio of 50:25:25, pH 7.24±0.20 and C: N ratio of 30:1, mean methane yield was maximum 99 mL/g. In contrast, other mixtures provided less methane, although C: N ratio was increased. Hence, co-digestion was carried out by maintaining a ratio of 50:25:25 of PWW: BCM: DCM and C/N of 30/1.

Table 4.6 Effect of PWW, DCM & BCM mixing ratio on methane production

Mixing %			**C/N ratio**	**pH**	**Mean methane yield (mL/g)**
PWW	**DCM**	**BCM**			
25	37	38	19/1	7.49±0.50	86
40	30	30	24/1	7.43±0.30	94
50	25	25	30/1	7.24±0.20	99
60	20	20	33/1	7.17±0.10	97
75	12	13	61/1	6.88±0.30	95

4.12 EFFECT OF OLR, HRT AND F/M RATIO ON REACTOR PERFORMANCE BOTH AT MESOPHILIC AND THERMOPHILIC CONDITIONS

The chemical and elemental composition of PWW, DCM, BCM and active inoculum is described in Table 4.7. To authenticate the bilateral effect of OLR and HRT on reactor performance under mesophilic and thermophilic conditions, reactors were ran at different HRT and OLR at individual condition as exposed in Tables 4.8 and 4.9. At the two temperature optima, the structures of the active microbial communities are quite different. The results projected in Figure 4.8 illustrates that reactor operated at thermophilic (55 ˚C) conditions produced higher biogas yield compared to that of mesophilic (37 ˚C). For instance, under thermophilic (55 ˚C) conditions reactor worked efficiently at 10 days HRT with an OLR of 6.31 kg COD/m^3 d achieving a 97.5 ± 1.00% COD reduction. Methane generation was found 0.46 m^3/kg COD d. Mentionable that, maximum methane generation was found 0.65 m^3/ kg COD d at 7 days HRT. The reason may be, lesser HRT will result in hydraulic overload which will avert the methane-producing bacteria from replicating swift enough avoiding washout of alkalinity. Moreover, abridged HRT will lessen the interaction time between waste and microbes (Poh and Chong, 2009 and Mourn et al., 2007).

Thermophilic reactors are capable to sustain greater OLRs and to run at abridged HRT generating extra biogas as well (Latif et al., 2011). On the contrary, COD removal rate was higher under mesophilic (37 ˚C) condition compared to thermophilic (55 ˚C) condition. Fang and Chung in 1999 achieved 84% of COD removal in mesophilic reactor whereas 69-83% in thermophilic reactor treating protinaceous wastewater treatment using 2.8 l UASB. The grade of comprehensiveness of biodegradation system can be strongly indicated by COD, as any unconsumed material will need oxygen to complete degradation (Ward et al., 2008).

Table 4.7 Chemical and Elemental composition of PWW, DCM, BCM and active inoculum

Parameters	PWW	DCM	BCM	Inoculum	
				Mesophilic	Thermophilic
Acetic (mg/L)	1020±20	125±15	58±10	50±6	30±6
Propionic (mg/L)	0	36±5	33±5	28±3	40±5
Isobutyric (mg/L)	0	40±5	105±4	22±4	26±3
Butyric (mg/L)	0	53±5	115±7	22±5	20±2
Total VFA (mg/L)	2210±20	324±6	375±5	3200±20	3000±20
TKN (mg/L)	44±5	1000±6	1200±150	1044±90	835±34
pH	6.12±0.2	7.10±0.23	7.43±0.4	7.15±0.03	6.98±0.10
TS (g/g)	0.30±0.05	0.145±0.05	0.107±0.06	0.026±0.01	0.024±0.01
VS (g/g)	0.46±0.02	0.115±0.02	0.085±0.04	0.021±0.01	0.020±0.01
COD (mg/L)	15000±30	35000±20	40000±20	6840±20	7700±20
TOC (mg/L)	4950±50	13000±1000	14000±1050	3900±50	4100±40
C/N ratio	107/1	13/1	11.6/1	-	-

Values are the mean + S.D. of the 3 determinations

Table 4.8 An anaerobic CSTR Bioreactor performance operated at (mesophilic condition) 37˚c at varying OLR and HRT under steady state condition

Parameters	HRT (d)				
	10	7	4	2.5	1.5
OLR (kg COD/m^3 d)	6.31	8.99	15.73	25.17	27.24
COD reduction (%)	98±0.5	95±0.6	95±0.5	95±1	48±3
BOD (g/L)	0.32±0.15	1.31±0.30	1.47±0.5	1.45±0.2	15.97±1
Methane production (%)	83.45	75.90	71.45	68.98	63.34
Total biogas production (m^3/m^3 d)	4.93±0.4	6.17±0.50	10.98±1	13.95±1	4.95±0.1
Total methane (m^3/m^3 d)	4.09±0.50	4.68±0.70	7.84±0.5	9.62±1	3.12±0.5
Methane yield (m^3/Kg COD d $_{added}$)	0.64	0.52	0.49	0.38	0.11
pH	7.5±0.50	7.3±0.60	7±0.50	7±0.50	3.58±1.5
VSS (g/L)	25.24±1	28.2±0.5	30.1±0.6	44.95±0.3	45.40±0.5
F/M (g COD/g VSS d)	0.25	0.31	0.52	0.56	0.6
Total VFA (mg/L)	154±1	178±2	201±1	233±1	275±2
Total alkalinity (as $CaCO_3$ mg/ L)	1000±200	950±150	780±130	720±150	680±130
VFA/ alkalinity	0.154	0.19	0.26	0.32	0.40

Values are the mean + S.D. of the 3 determinations

While batch co-digestion of vegetable waste and wooden chips, fast deprivation of fatty acids was observed at 55˚C compared to 38˚C, and 95% of methane generation was realized at the end of 11 days of thermophilic conditions compared to 27 days of mesophilic conditions (Hedge et al., 2007). Nevertheless, it is obvious that at highest OLR (27.24 kg COD/ m^3 d), while food to microorganism ratio was 0.6 g COD/g VSS d, the process was stable (Table 4.8). It is a must to be noticed that an enhancement in methane generation from thermophilic system has to be well-adjusted to the enhanced energy demand for running the reactor at greater temperature. Therefore, the best performance was identified with mesophilic conditions in terms of stability and waste water stabilization (Table 4.8).

Table 4.9 An anaerobic CSTR Bioreactor performance operated at (thermophilic condition) 55˚c at varying OLR and HRT under steady state condition

Parameters	HRT (d)				
	10	7	4	3	2.5
OLR (kg COD/m^3 d)	6.31	8.99	15.73	20.97	25.17
COD reduction (%)	97.5±1	94±1.0	82±1	77.30±3	55±1.0
BOD (g/L)	0.47±0.1	0.59±0.10	2±0.05	7±1.0	8±1.0
Methane production (%)	64.67	45	60	50	31
Total biogas production (m^3/m^3 d)	7.5±2	13.5±1.5	8.5±1	4.5±0.3	1.7±0.3
Total methane (m^3/m^3 d)	4.8±0.8	6.08±0.70	5.1±0.5	2±0.50	0.53±0.3
Methane yield (m^3/Kg COD d $_{added}$)	0.76	0.68	0.32	0.10	0.02
pH	7.55±0.6	7.39±0.50	7.36±0.4	7.22±0.5	5.45±0.5
VSS (g/L)	21.75±0.5	25.68±0.6	29.12±1	36.16±1	41.95±3
F/M (g COD/g VSS d)	0.29	0.35	0.54	0.58	0.6
Total VFA (mg/L)	109 ±1	80±2	136±1	142±2	158±2.0
Total alkalinity (as $CaCO_3$ mg/ L)	540±70	510±40	478±50	445±50	395±40
VFA/ alkalinity	0.20	0.15	0.28	0.32	0.40

Values are the mean + S.D. of the 3 determinations

4.13 EFFECT OF pH AND VFA: ALKALINITY RATIO

The bacteriological species of anaerobic digesters is sensible to pH variations, and methanogenesis is disturbed greatly (Leslie et al., 1999). The effect of feed pH (6.02) on reactors efficiency at different conditions was taken into account, and reactors performed best at HRT of 2.5 days and temperature of 37 °C. Effluent alkalinity and pH were recorded at 720 ± 150 mg/L $CaCO_3$ and 7 ± 0.5, correspondingly. The alkalinity of effluent while reactors running under mesophilic or thermophilic conditions never exceeded 1 g/L, stayed between 400 ± 50 to 700 ± 50 mg/L $CaCO_3$. Bicarbonate alkalinity in the range 2.5–5 g/L provided sufficient buffering capacity so as to formation of excessive volatile acids resulted in solitary a negligible pH reduction.

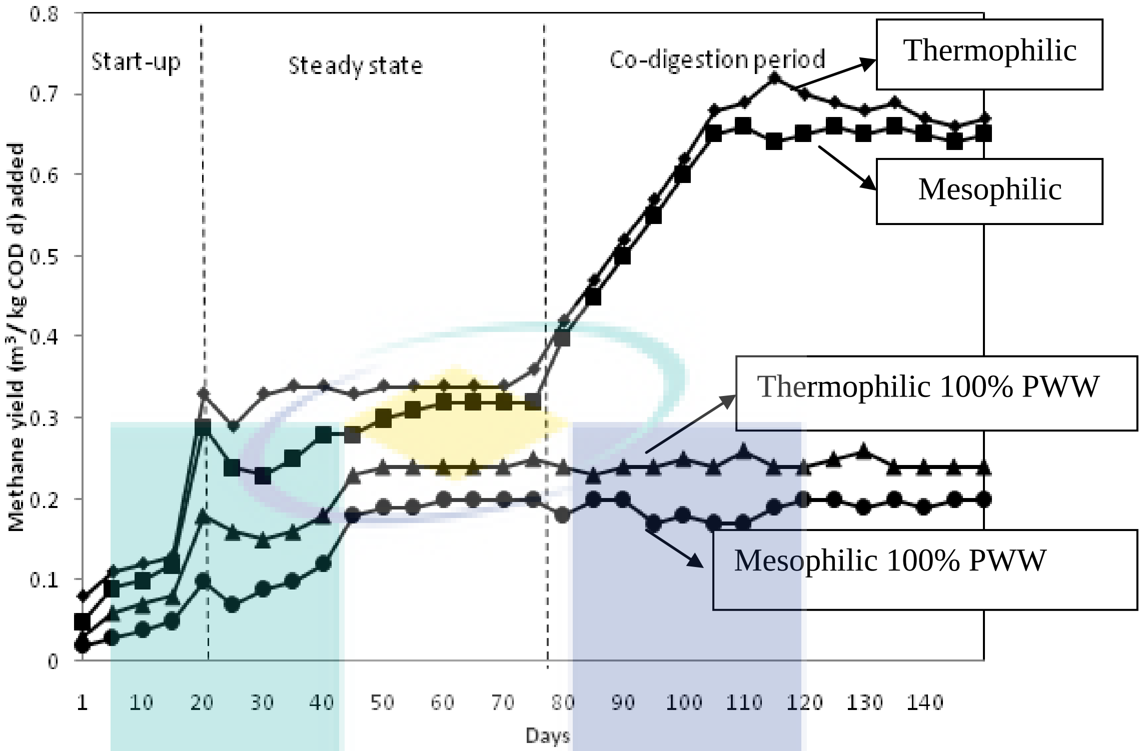

Figure 4.8 Evaluation of Methane production m3/ kg COD d for the mesophilic and thermophilic systems, where ♦-CH_4 yield at PWW:BCM:DCM at 50:25:25 at 55 °C, ■- CH_4 yield at PWW:BCM:DCM at a ratio of 50:25:25 at 37 °C, ▲- CH_4 yield at 100% PWW at 55 °C, ●- CH_4 yield at sole PWW at 37 °C

The reactors working under mesophilic conditions exposed alkalinity of 780 ± 130 $CaCO_3$, mg/L & pH of 7± 0.5 above critical HRT 2.5 d. Reactors working under thermophilic conditions exposed alkalinity of 400 ± 70 $CaCO_3$, mg/L & pH 7.0 ± 0.5 at a critical HRT 3 d. The VFA/alkalinity ratio remained in the range 0.1–0.4, which revealed stability of AD. At a VFA:alkalinity ratio <0.4, the reactor can be considered stable, as reported by Callaghan et al (2002). Nevertheless, Tables 4.8 and 4.9 showed that there was no drastic drop of pH below and above the critical HRT for both conditions. Thus, co-digestion of PWW with BCM and DCM can successfully sustain sufficient buffering capacity without extraneous inclusion of nutrients and buffering sources restricted by need of nitrogen. Conversely, BCM comprises a great amount of ammonia that can inhibit methanogenesis. Hence, co-digestion of PWW with DCM and

BCM might decrease both chemical application and operating charges for methane generation from PWW. Likewise, effluent of co-digestion incorporated minor quantity of ammoniacal nitrogen compared to that of PWW alone. It could determine inhibitory influence of ammonia on AD and lead a subsequent-treatment process for nitrogen elimination easy and inexpensive. Thus, co-digestion might accomplish more profitable biogas production.

4.14 VFA ACCUMULATION, C: N RATIO AND METHANE PRODUCTION POTENTIAL

COD degradation or biogas generation is introverted by the buildup of fatty acid, which often makes system unbalanced and leads to slurry washout. Co-digestion using manure assists in enhancing buffering capacity and offers a nitrogen resource for bacterial synthesis which will cause an established AD system. Results showed that VFA reduced from approximately 275 to 154 mg/L under mesophilic conditions and to almost non-measurable stages under thermophilic conditions at the time of digestion. Figure 4.9 shows that mutually for mesophilic and thermophilic digestion, the reduction of VFAs were differently.

Slow VFA reduction was detected in the case of mesophilic digestion. Additionally, after 21 days minor VFAs were present within the effluent about 154 mg/L. At the same time at thermophilic temperature, VFAs were swiftly used up and about 33% of those detectable after day 10. The variation in rate of VFA consumption between the two different temperatures was possibly due to the consequence of immense action of microbes at thermophilic conditions (Aoki and Kawase, 1991). Nonetheless it might also be an influence of specialized microbes developing at greater temperatures (Kim et al., 2002). In comparison, when reactors were fed with PWW alone under both mesophilic and thermophilic temperatures, >3 g/L VFA was accumulated, indicating reactor failure. The methanogenic pH limit (6.8–7.5) was maintained to avoid the prevalence of the acid-forming microorganisms that might form VFA agglomeration.

For all OLRs and HRTs, the total VFA concentration remained low (100–275 mg/L), with no VFA accumulation (Table 4.8 and Table 4.9).

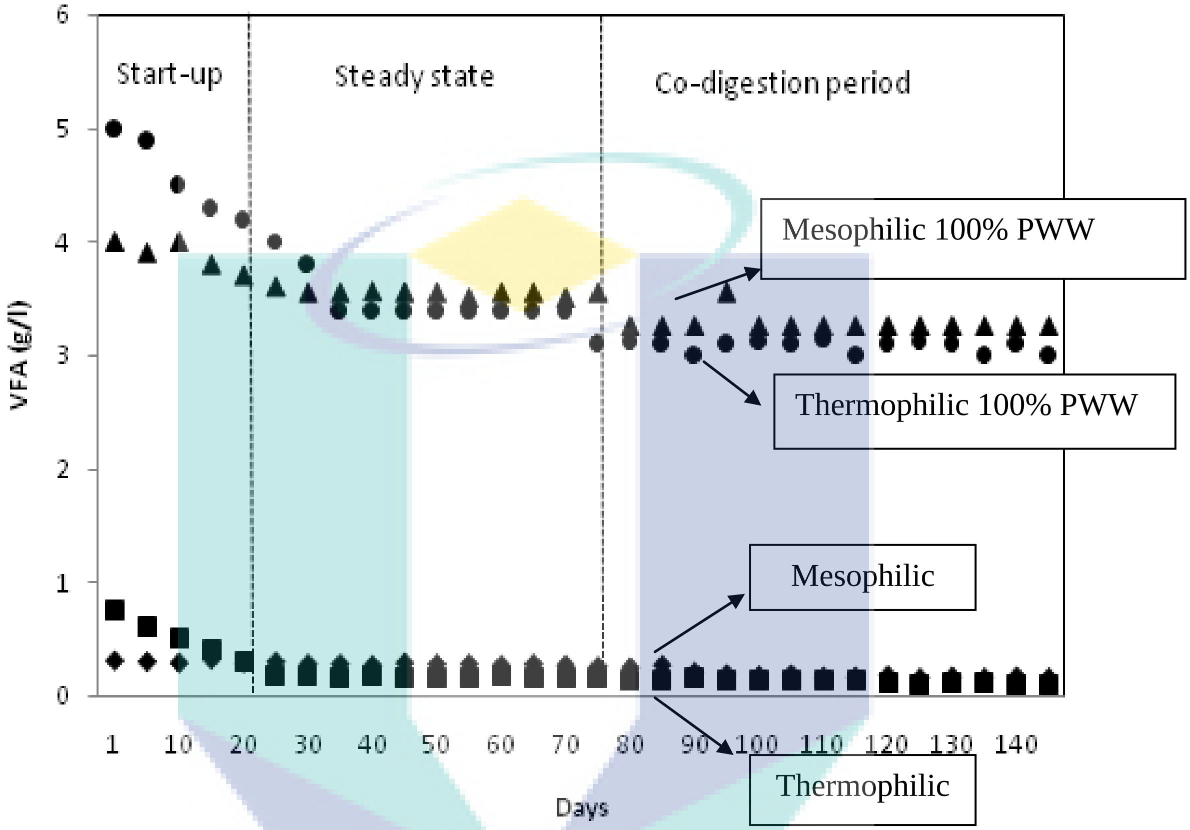

Figure 4.9 Evaluation of VFA accumulation vs. time, where, ▲- VFA at 100% PWW at 37 °C, ●-VFA at 100% PWW at 55 °C, ♦- VFA at PWW:BCM:DCM at a ratio of 50:25:25 at 37 °C, ■- VFA at PWW:BCM:DCM at 50:25:25 at 55 °C

In current work, the mesophilic co-digestion of PWW, DCM & BCM indicated an encouraging methane production of 50–60%/kg COD added, while thermophilic digestion showed 50–65% compared with PWW digestion alone (Figure 4.8). In 2010, Goberna et al. have reported similar results in which integrated digestion of cattle manure and olive mill wastes produced 179 mL CH_4 /gVS $_{added}$ in case of mesophilic digestion, whereas a 17% increase in methane production was found at thermophilic. Additionally, Gelegenis et al. (2007) have shown that addition of 25% olive mill wastewater to mesophilic AD of diluted poultry manure, increased biogas production by 20–25%, probably for counterpoise of nutrients internal feed mixes and maintenance of low TS levels. Co-digestion of sewage sludge and agricultural waste may increase the methane generation of AD system (Goberna et al., 2010). Hartmann and Ahrin have shown in 2005 that co-digestion of cattle manure and municipal solid waste also enhances methane generation.

To ameliorate C: N ratio up to the optimal to accelerate methanogenesis is one aim of co-digestion technology. The C/N ratio is a substantial issue that could be the restrained in AD. During current study, the maximum methane generation of 99 mL/g was attained when C/N ratio was 30/1 (Table 4.6). It is generally agreed that microbes exploit carbon 25–30 folds more than N. Hence, to apt this demand, microorganisms require a 20–30/1 ratio of C/N, with highest quantity of carbon being freely degradable (Bardiyn et al., 1997).

4.15 OPTIMAL SUBSTRATE COMPOSITION IN CO-DIGESTION EXPERIMENTS

The concept of co-digestion can be defined as the instantaneous digestion of uniform blend of dual or more wastes. Before AD it is essential to do a comprehensive characterization of wastewaters that shall undergo treatment because physicochemical individuality's of them play a decisive part as it intensely impacts the outcomes achieved in AD. Hence, mean outcomes for essential parameters of PWW, AM and co-digestion wastes by three-layered analysis of tasters are presented in Table 4.10. PWW and AM show contrasting performance concerning definite physicochemical properties.

In particular, AM showed greater pH index, biological plaint and alkalinity (i.e. greater buffering capacity) than PWW. Yet, C/N proportion in PWW surpasses that of AM and offers a value adjacent to that informed by Hashimoto, 1981.

All these may recommend that the mutual degradation of both wastes should favor AD, as mix waste establishes an advantageous balance of discrete snags. As concerns to ACD, a steady decline of pH, COD and alkalinity are detected as PWW concentration is elevated in mixing proportion, thus, these outcomes recommend that the sample that shall attain the extreme methane shall be an ACD mixture. To measure the influence of the digest ate concerning ecological, active and fiscal aspects, numerous AD of PWW, AM and their mixture, using different organic loadings, was examined. The content of input samples ranged from 0% PWW (i.e.100% AM) to100% PWW (0% AM) with 10% PWW stepping increment.

Table 4.10 Physicochemical characterization of substrates

	AM	**10/90**	**20/80**	**30/70**	**40/60**	**50/50**	**60/40**	**70/30**	**80/20**	**90/10**	**PWW**
pH	7.57±0.4	7.52±0.35	7.44±0.34	7.39±0.33	7.32±0.3	7.27±0.3	6.98±0.28	6.83±0.25	6.59±0.23	6.31±0.2	6.16±0.2
COD total (g/L)	40±0.65	37.5±0.65	34.96±0.65	31.95±0.6	28.91±0.6	25.86±0.6	22.75±0.6	19.66±0.5	17.6±0.5	16.56±0.5	15.51±0.6
BOD_5 (g/L)	7.91±0.38	7.91±0.36	7.9±0.36	7.89±0.36	7.89±0.35	7.87±0.35	7.87±0.35	7.86±0.3	7.84±0.3	7.81±0.3	7.8±0.3
VFA (g/L)	0.38±0.05	0.41±0.06	0.51±0.08	0.71±0.1	0.91±0.1	1.3±0.12	1.36±0.14	1.61±0.14	1.81±0.15	2.1±0.15	2.3±0.15
Alkalinity (g/L)	0.77±0.1	0.76±0.1	0.75±0.1	0.74±0.1	0.73±0.1	0.73±0.1	0.73±0.1	0.72±0.1	0.71±0.09	0.71±0.09	0.7±0.09
TN (g/L)	1.2±0.5	1±0.4	1±0.4	0.9±0.3	0.8±0.3	0.3±0.2	0.3±0.2	0.25±0.2	0.2±0.15	0.09±0.01	0.046±0.01
TOC (g/L)	14±1	13±0.9	12±0.8	11±0.7	10±0.5	9±0.5	8±0.5	7±0.45	6±0.4	5±0.2	4.95±0.2
C/N ratio	11.6/1	13/1	12/1	12.22/1	12.5/1	30/1	26/1	28/1	30/1	55.55/1	107.1/1

Table 4.11 Outcomes of ACD trials in CSTR

	PWW	**90/10**	**80/20**	**70/30**	**60/40**	**50/50**	**40/60**	**30/70**	**20/80**	**10/90**	**AM**
Flow (mL/day)	91	74	64	171	186	191	206	241	251	256	256
HRT (days)	15	14	13	12	11	10	9	8	7	6	5
pH	7.51±0.1	7.41±0.1	7.36±0.1	7.31.±0.1	7.29±0.1	7.26±0.04	7.21±0.04	7.16±0.03	7.11±0.03	6.91±0.25	6.51±0.2
VFA (g/L)	0.61±0.15	0.61±0.15	0.61±0.15	0.61±0.14	0.6±0.14	0.6±0.12	0.51±0.1	0.51±0.1	0.51±0.1	0.41±0.01	0.31±0.1
Alkalinity (g/L)	0.25±0.1	0.25±0.1	0.24±0.1	0.24±0.1	0.23±0.1	0.22±0.05	0.21±0.05	0.19±0.05	0.18±0.05	0.17±0.05	0.13±0.1
COD reduction (%)	98.59±2	98.56±2	98.54±2	98.53±1.5	98.51±1	98.5±1.5	96.1±1.5	94.1±1.5	60.1±1	58.1±1	44.1±1
Methane (%)	83	79	76	73	71	80	69	66	64	63	61
Total biogas (m^3 m^{-3} d^{-1})	6.1±0.4	7.1±0.5	9.1±0.6	10.1±0.7	12.1±0.8	14.1±1	13.1±0.9	11.1±0.8	9.1±0.7	6.1±0.6	5.1±0.5
CH_4(m^3 m^{-3} d^{-1})	4.93±0.4	5.47±0.6	6.76±0.6	7.3±0.7	8.5±0.7	11.07±0.7	8.85±0.6	7.16±0.6	5.68±0.5	3.73±0.4	3.1±0.4

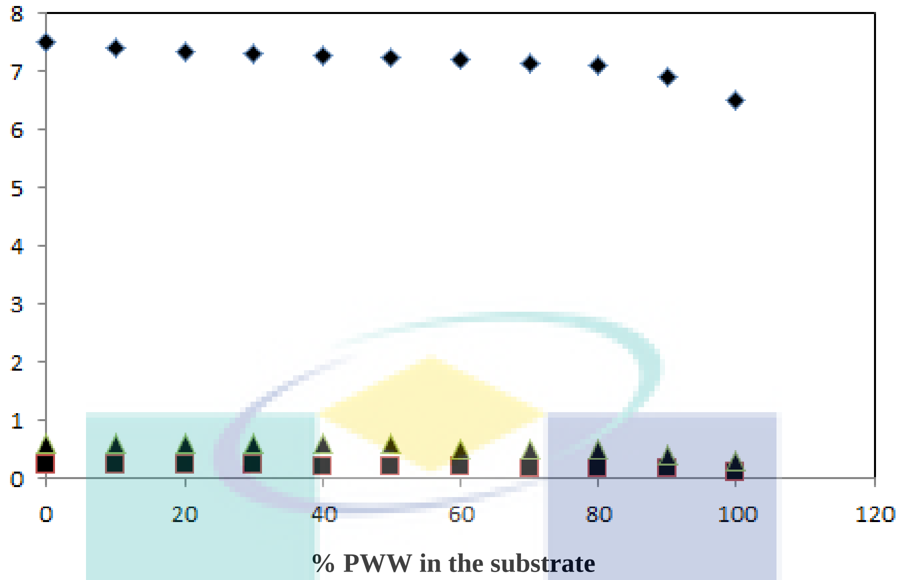

Figure 4.10 Role of the waste proportion in the regulator parameters: ♦- pH, ▲-VFA (g/L) and ■-alkalinity (g/L).

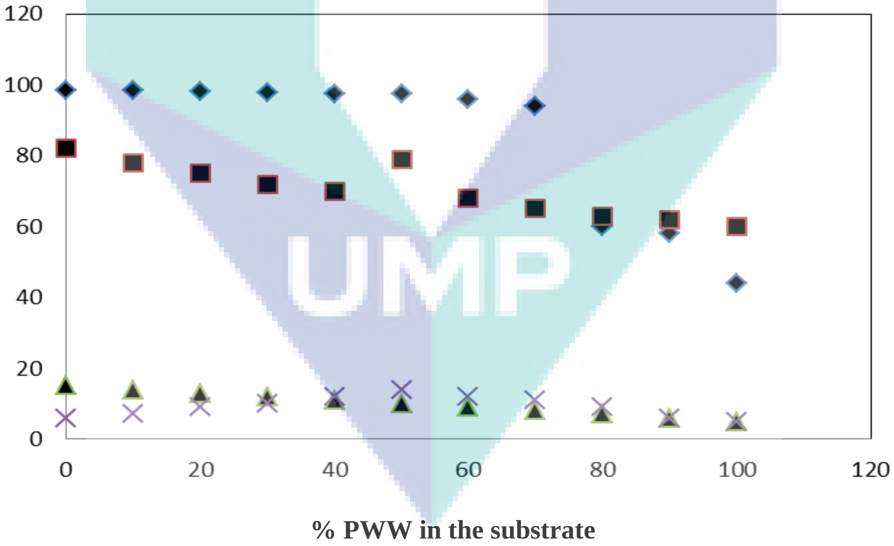

Figure 4.11 Role of waste proportion in ecological and energy parameters: ♦-COD reduction (%), ■- methane (%), ▲-HRT (d) and X- total biogas (m^3 m^{-3} d^{-1})

The outcomes equivalent with the optimum organic loading that reach the maximum methane generation and COD destruction, are exclusively displayed in Table

4.11. Besides, the concrete data permit seeking the influence of mixing proportion of wastewater on ACD and studying the latent of individual proportion in producing methane and reducing COD that can be achieved. The act of wastewater combination in the ACD controller parameters (pH, alkalinity and VFA) is presented in Figure 4.10. As seen, the pH of ACD medium was maintained from 6.5 to 7.5 during investigating the influence of various mixing proportion of PWW and AM. Eventually, the maximum standards were grasped with ACD proportion 50% PWW /50% AM. However, pH was detected to be within a tiny interval of 6.5–7.5 during entire set of trials so that the buffering capacity of the ACD medium may resist sudden alterations of pH and maintain it in the optimum limit for growth of methanogenic microbes (Lü et al., 2013). Besides, Figure 4.10 explains that VFA (g/L) and alkalinity (g/L) followed a steady declining pattern as percentage of PWW was elevated in the wastewater samples. This is observed may be through co-digestion that produces the balanced buffering capacity to make the conversion faster from acidogenesis to methanogenesis to supply methane as a last by-product. Furthermore, PWW are less decomposable than AM as per biodegradability index BOD_5/COD of AM is 0.19 as compared with 0.51 of PWW that specifies a less organic ingredients of biodegradable substance. Similarly, the steady decline of VFA is interpreted: if a lesser fraction of samples are hydrolyzed, a lesser VFA may be produced at acetogenic phase that may permit a calmer digestion of this element at methanogenic phase. As the outcomes explained yet, it may be noted that all wastes yield achieved biodegradation as the regulator parameters values comply with those of optimal standards for the growth of each kind of microbes responsible for ACD. Some statistics on ecological and potential aspect parameters of ACD with PWW and AM are set onward in Figure 4.11. As PWW own lesser biodegradable latent, the drop of COD in ACD was observed to reduce with the increase in PWW proportion in mixture. Likewise, the maximum degradation were attained up to 98.59% for 90% PWW /10% AM at 15d HRT. In particular, 98.5% COD degradation was achieved for 50% PWW /50 %AM at 10d HRT. A 5 days HRT reduction can be observed for almost the COD removal efficiency. However, the bottommost standards of COD elimination were within 58% to 44% from 10% PWW/ 90% AM and 100% AM at 6d and 5d HRT (Figure 4.11). As AD is a bio-digestion method, this is not expected to provide a complete eradication of pollutant. Towards this, the above projected outcomes favor ACD as an ecologically apposite and feasible procedure for digestion of PWW, AM and their combinations. Peak amounts of biogas generation were obtained from 14 to 15 m^3 /m^3d and resembled to the bio-digestion of PWW and waste combinations primarily consist of this kind of waste (Figure 4.12). Nevertheless, the greatest methane generation was observed up to 11 m^3 / m^3d for the specific combination 50% PWW/ 50% AM, whereas the minimum rate was 4.92 m^3 / m^3d respectively achieved for 100% PWW at 10 d HRT (Figure 4.12). Eventually, HRT of wastes indicated a falling tendency as the magnitude of AM in the mixture was augmented.

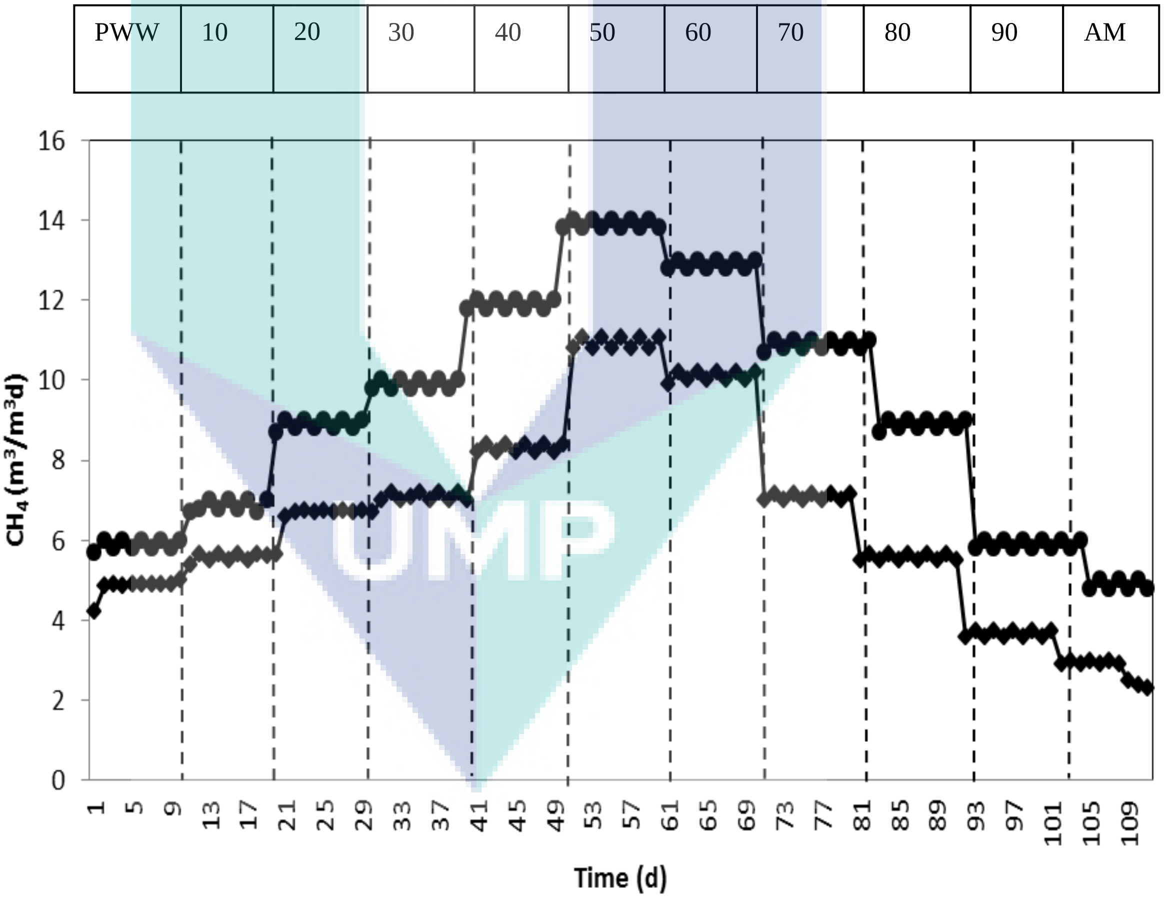

Figure 4.12 Relative biogas and methane generation during treatment period, where ●- biogas; ▲- methane.

Nonetheless, most suitable HRT was achieved up to 10 d for higher degradation of wastewater although abridged HRT had an inverse influence on digestion. The extreme decline was understood to occur for waste proportion 50% PWW / 50% AM, although the minimal HRT were achieved 2-1d for both 10% PWW/ 90% AM and AM waste compositions. The contraction of the asking period to degrade a certain amount of waste is ambiguous joining with the intensification of suckling flow. It is caused by the fall of BOD_5/COD proportion linked to AM: a minor magnitude of waste of the filling into the digester should be digested and a greater filling flow should consequently be desired to supply the optimum biological charge. HRT is taken as a concerned regulating parameter throughout the planning process of a manufacturing-scaled AD setup. It shows a great influence on construction costs provided that the higher the value of the HRT, the larger the volume of the anaerobic reactor. According to the outcomes all kinds of wastes under this work were fit for AD, yet PWW were detected to exhibit minor yield attainments. The supplementation of a certain portion of PWW to AM may therefore be a sensible method. Figure 4.13 showed that mixing ratio of 50% PWW/ 50% AM was significantly different from others. Hence, 50% PWW/ 50% AM was noted as the optimum proportion, given the resulting data was attained for that specific sample combination: (i) greater COD, pH and alkalinity of digestion medium (ii) approximately threefold methane generation gained by ACD of PWW and AM. When the prime waste proportion for ACD was detected, the ecological, lively and fiscal feasibility's of the same, along with those of control ones are next deliberated. Nonetheless, Figure 4.14 and 4.15 explain that MLVSS for mix proportion of 50% PWW / 50% AM and 100% PWW respectively were steadily declined towards the end of this work. It may be attributed to as huge OLR with abridged HRT that was inadequate to permit stabilizing of biomass. However, pH change was quite different for 50% PWW / 50% AM and 100% PWW waste proportion. A drastic drop of pH from 7.4 to 5 was observed for mono-digestion of PWW in Figure 4.10 although co-digestion with proportion 50% PWW / 50% AM provided stable pH. Looking at Figures 4.14 and 4.15, it is noticeable that at each different OLR, MLVSS increased sluggishly and steadied for a limited day having a trivial reduction below the former value that signifies the biomass amplified and then steadied. This may be caused by acclimation of the microbes to different states (Panorama, 2011).

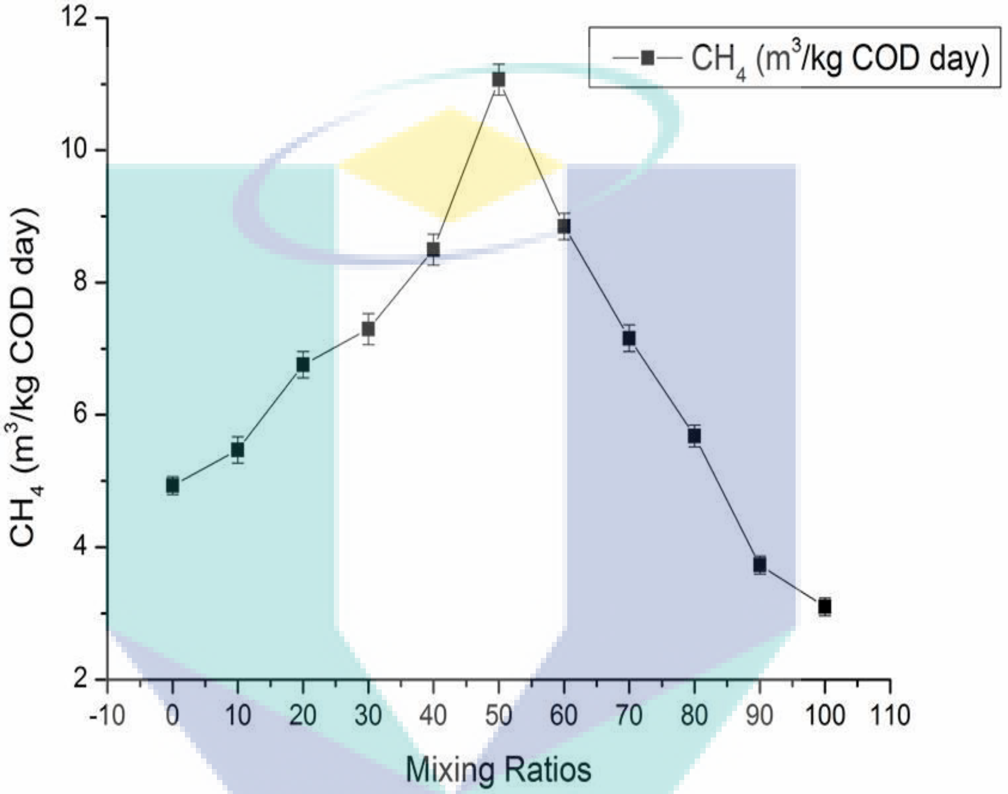

Figure 4.13 Mean methane generation for different mixing ratios of PWW and AM, where ■- methane.

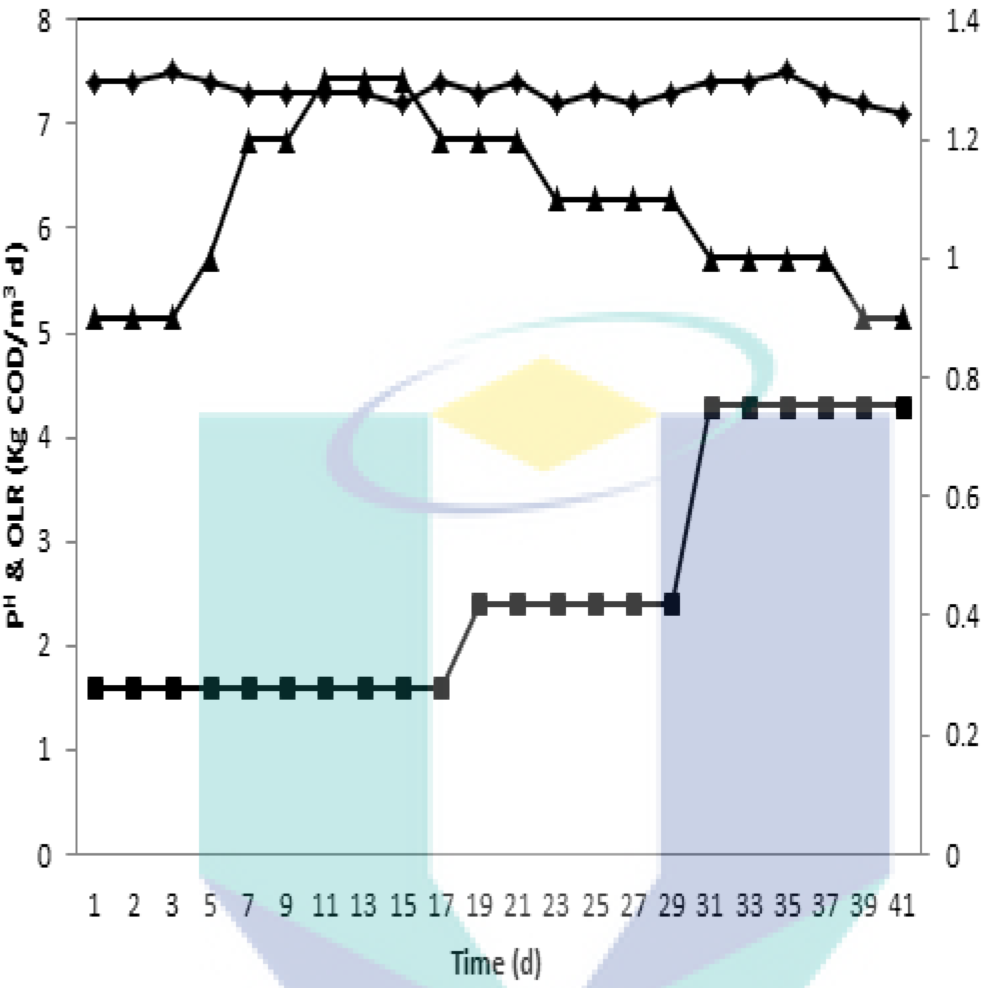

Figure 4.14 The connection among pH, mixed liquor volatile suspended solids and organic loading rate vs. process time when proportion was 50% PWW/ 50% AM, where ♦- pH, ▲-MLVSS and ■-OLR.

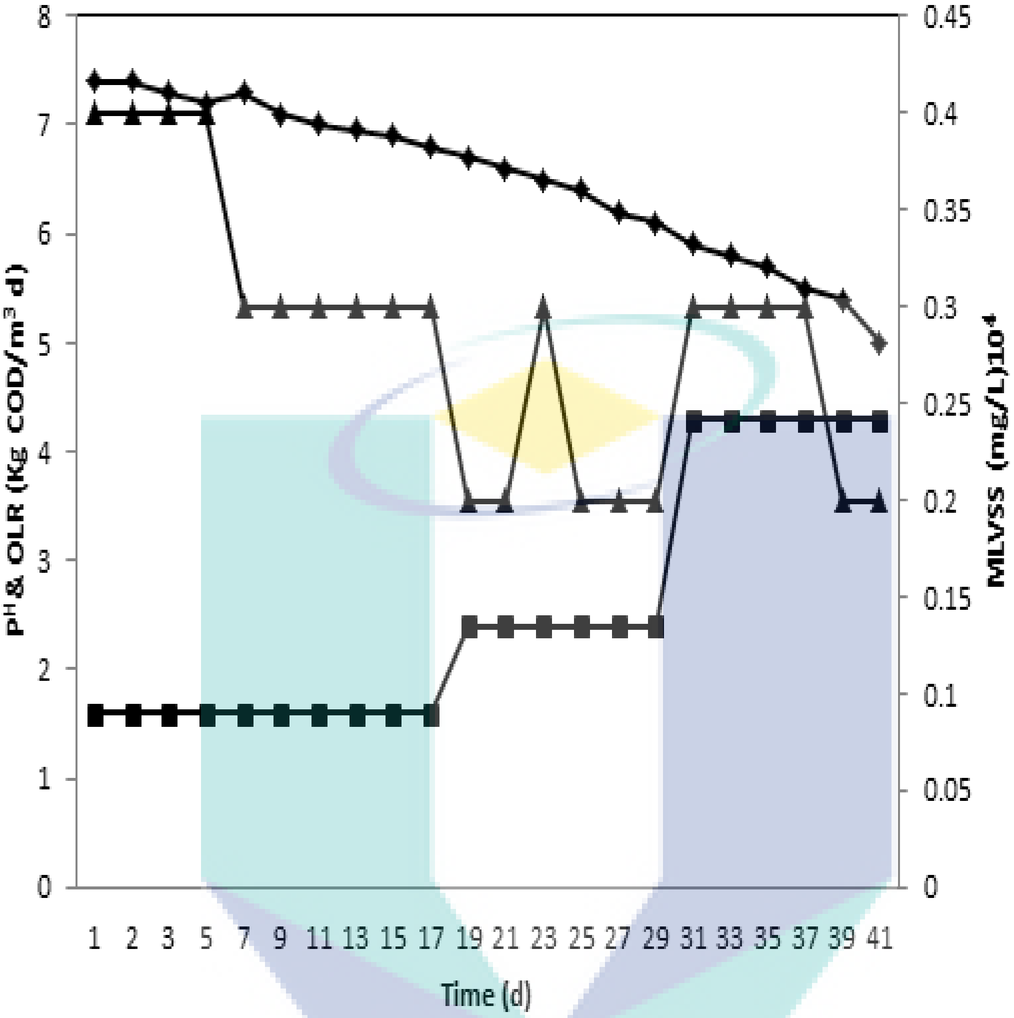

Figure 4.15 The connection among pH, mixed liquor volatile suspended solids and organic loading rate vs. process time when proportion was 100% PWW/ 0% AM, where ♦- pH, ▲-MLVSS and ■-OLR.

4.16 OPTIMUM OPERATING FLOW RATE

In order to find the optimum operating flow rate, it was essential to calculate the organic digestion rate after completion of each trial. Noticeably, considering this parameter entirely, the least flow rate, 170 mL/day was found to be the optimum. The methane production was also seen to be higher at this flow rate. A highest biogas generation of 580 mL/h was attained from Table 4.12. It was found handy from the energy point of view, and for digestion the attained COD_f was too low during achieving the steady condition.

Tables 4.12 and 4.13 indicate that with a gradual increase in the flow rate of the trials, there is a decrease in the microbial community (expressed by volatile solids), which may be detected because of the increase in alkalinity. Subsequently, it caused inhibition to the bio-methane generation for the gradual reduction in the microbial community took part in the degradation process and, thus, a decrease in suspended solids.

Table 4.12 Reactor outcomes for different experimental trials

Trial	**Flow rate (mL/d)**	**Retention time (d)**	**COD_f(g/L)**	**Biogas (mL/h)**	**TN (g/L)**	**TOC (g/L)**
1	170	15	9±0.8	580±16	0.86±0.05	0.77±0.04
2	220	13	9.4±0.8	550±15	0.94±0.06	0.82±0.05
3	300	9	10±0.9	520±15	0.99±0.06	0.83±0.05
4	370	9	10.5±0.9	480±14	1.08±0.06	0.84±0.05
5	410	8	11±1	465±14	1.10±0.07	0.85±0.06
6	475	8	11.5±1	440±14	1.15±0.07	0.86±0.06
7	540	8	12±1	410±12	1.25±0.08	0.87±0.06
8	600	7	13±1.2	402±12	1.38±0.09	0.88±0.07
9	640	7	14±1.3	390±12	1.47±0.09	0.89±0.07
10	680	7	15±1.4	330±10	1.6±0.09	0.90±0.07

Values are the mean + S.D. of the 3 observations

Table 4.13 Reactor outcomes for different flow rates

Trial	**Flow rate (mL/d)**	**BOD_5 (g/L)**	**VFA (g/L)**	**Alkalinity (g/L)**	**VS (g/L)**
1	170	3.60±0.3	0.49±0.03	5.30±0.3	3.30±0.3
2	220	3.95±0.3	0.63±0.04	5.50±0.3	3.00±0.3
3	300	4.40±0.4	0.75±0.04	5.80±0.4	2.75±0.3
4	370	4.90±0.4	1.01±0.05	6.10±0.4	2.50±0.2
5	410	5.45±0.5	1.30±0.05	6.70±0.4	2.20±0.2
6	475	5.80±0.5	1.70±0.06	7.15±0.5	2.00±0.2
7	540	6.30±0.6	2.20±0.06	7.50±0.5	1.75±0.2
8	600	6.75±0.6	2.70±0.07	8.10±0.6	1.50±0.2
9	640	7.35±0.7	3.40±0.07	8.60±0.6	1.36±0.1
10	680	7.8±0.7	5.70±0.07	8.98±0.7	1.20±0.1

Values are the mean + S.D. of the 3 observations

In order to find the optimum operational parameters, and more precisely for the determination of the flow rate, it is obligatory to assess the bio-digestion attained after the accomplishment of each trial. If the attained values of degraded COD are considered, it is reasonable to state that the most suitable flow rate for highest purification would be the lowest that is 170 mL/day (see Table 4.12).

Nevertheless, from the financial point of view, the energy return or the generation of biogas might make it more cost-effective to select a flow rate. The highest generation of biogas was found to be approximately 580 mL/h corresponding to a flow rate of 170 mL/day. The selection of the optimum flow rate as well as with other parameters will be justified in the succeeding sections.

The key parameters involved in this process will be evaluated in order to find the optimum flow rate:

a) Highest organic digestion. The digestion of the ultimate substrates was measured in terms of the degraded COD. Noticeably, the environmental guidelines to permit to discharge in the sewage system are compiled by the flow rates of 170, 220, 300 and 370 mL/day. For the environmental compliance of that of the higher flow rates, it might be required to use another degradation process, like a succeeding aerobic digestion. Consequently, based on the feature of the rate of degradation, (i) trials 5–10 (flow rates of 410, 475, 540, 600, 640 and 680 mL/day) might have to be excluded as not complying with the environmental guidelines, and (ii) any of the trials 1-4 (flow rates 170, 220, 300, and 370 mL/day) might be selected, as they comply with the discharge limits. In particular, trial 4 with flow rate of 370 mL/day may be selected as the optimum, since this is the highest flow rate and provides adequate treatment at the shortest retention time. Furthermore, it commercially means a tiny digester, and therefore, proves better feasibility of the application due to lower financial costs. b) Yield parameters. Eleven experimental trials for yield parameters have been listed in Table 4.14. Among the four experimental trials that complied with the environmental guidelines discussed in the earlier part of this work, that corresponding to the flow rate of 170 mL/day was the optimal value. Interestingly, at this particular flow rate a higher methane generation was observed when compared with experimental trials 1, 2 and 3 (Table 4.14).

Table 4.14 The anaerobic co-digestion yield (50% PWW/50% AM) for different experimental trials

Trial	Flow rate (mL/d)	CH_4 (m^3/kg)	VSS (kg /kg)	SMA $mLCH_4$/gVSS day
1	150	0.62±0.05	0.65±0.03	169±0.5
2	170	0.7±0.05	0.65±0.03	175±0.5
3	220	0.65±0.05	0.56±0.03	170±0.5
4	300	0.60±0.05	0.48±0.03	163±0.5
5	370	0.55±0.04	0.42±0.02	157±0.5
6	410	0.51±0.04	0.36±0.02	145±0.5
7	475	0.46±0.04	0.31±0.02	139±0.4
8	540	0.42±0.04	0.25±0.01	123±0.4
9	600	0.35±0.03	0.18±0.01	100±0.4
10	640	0.31±0.03	0.1±0.01	81±0.3
11	680	0.29±0.03	0.1±0.01	70±0.3

Values are the mean + S.D. of the 3 observations

Figure 4.16 showed that flow rate of 170 mL/day was significantly different from others. c) Energy assessment. The probability of the energy assessment of the biogas generated by an anaerobic reactor of PWW will be analyzed in this part.

Based on the optimum Figures interpreted above, for the value of ten different flow rates, digested COD values are: 0.225, 0.211, 0.196, 0.187, 0.170, 0.168, 0.165, 0.159, 0.150 and 0.144 kg. Such amount of energy may be utilized in a boiler to heat variety of liquids. Furthermore, it can also be used in an internal combustion engine installed to an electric generator to generate electrical energy (Table 4.15).

Table 4.15 Energy assessment at the startup period of co-digestion

Trial	Flow rate (mL/d)	Degraded COD (kg)	Biogas (m^3)	Biogas (m^3/kg)	Biogas (m^3/d)	Energy (Wh/d)
1	170	0.225±0.03	0.211±0.03	0.938±0.06	16.335±0.9	133.95
2	220	0.211±0.03	0.185±0.03	0.877±0.06	16.246±0.9	133.22
3	300	0.196±0.02	0.157±0.02	0.801±0.06	15.982±0.8	131.05
4	370	0.187±0.02	0.138±0.02	0.770±0.05	15.876±0.8	130.18
5	410	0.170±0.02	0.144±0.02	0.738±0.05	14.112±0.8	115.72
6	475	0.168±0.02	0.130±0.02	0.723±0.05	13.988±0.7	114.70
7	540	0.165±0.02	0.122±0.02	0.719±0.04	13.113±0.7	107.53
8	600	0.159±0.02	0.115±0.01	0.703±0.04	8.231±0.7	67.49
9	640	0.150±0.01	0.093±0.01	0.620±0.04	7.692±0.6	60.41
10	680	0.144±0.01	0.087±0.01	0.604±0.04	7.367±0.6	60.07

Values are the mean + S.D. of the 3 observations

The flow rates up to 370 mL/ day took sufficient time to degrade PWW, and thus yield more bio-methane.

The energy assessment for different experimental trials with a constant retention time of 10 d (Cao and Pawłowski, 2012; Tian et al., 2014 and Harsono et al., 2014) is listed in Table 4.15. This assessment was worked out by assuming that the resulted amount of biogas was that referring to the hourly generation by the retention time. It is noticeable that the most suitable flow rate might be 170 mL/day, as it provides the maximum energy generation. As it has been noticed earlier, the rest of the flow rates (Hwang et al., 2010) higher than 370 mL/day (particularly, 410, 475, 540, 600, 640 and 680 mL/day) needed to be excluded, since they were not attaining the organic digestion limit required for the substrates to be discarded in the sewage system. The biogas generation of the same is presented in Table 4.16. So, analyzing from the environmental point of view 170 mL/day might be the optimum flow rate as it provided the highest COD degradation.

The biogas generation of anaerobic co-digestion of PWW and AM is presented in Table 4.17. So, analyzing from the economical point of view 170 mL/day might be the optimum flow rate as it provided the highest COD degradation and methane production.

Table 4.16 Energy assessment at steady state period

Trial	Flow rate (mL/d)	Degraded COD (kg)	Biogas (m^3)	Biogas (m^3/kg)	Biogas (m^3/d)	Energy (Wh/d)
1	170	0.225±0.03	0.245±0.05	1.484±0.06	32.878±1	269.60
2	220	0.211±0.03	0.232±0.04	1.381±0.06	32.799±1	268.95
3	300	0.196±0.03	0.223±0.04	1.311±0.06	32.677±1	267.95
4	370	0.187±0.02	0.209±0.04	1.188±0.05	32.527±1	266.72
5	410	0.170±0.02	0.171±0.03	1.118±0.05	28.612±1	234.62
6	475	0.168±0.02	0.161±0.03	1.073±0.05	20.689±1	169.65
7	540	0.165±0.02	0.151±0.03	1.066±0.05	14.223±0.9	116.63
8	600	0.159±0.02	0.149±0.03	0.867±0.05	9.721±0.6	79.71
9	640	0.151±0.02	0.135±0.03	0.706±0.04	6.224±0.5	51.04
10	680	0.144±0.02	0.125±0.03	0.511±0.04	5.988±0.5	49.10

Values are the mean + S.D. of the 3 observations

Table 4.17 Energy assessment for ultimate period

Trial	Degraded COD (kg)	Biogas (m^3)	Biogas (m^3/kg)	Biogas (m^3/d)	Energy (KWh/d)
1	0.225±0.03	0.245±0.05	1.484±0.06	3259.13±50	26724.87
2	0.211±0.03	0.232±0.04	1.381±0.06	3047.69±50	24991.06
3	0.196±0.03	0.223±0.04	1.311±0.06	2836.25±40	23257.25
4	0.187±0.02	0.209±0.04	1.118±0.05	2556.78±40	20965.60
5	0.170±0.02	0.171±0.03	1.073±0.05	2240.12±40	19446.38
6	0.168±0.02	0.161±0.03	1.066±0.05	1945.32±40	18230.98
7	0.165±0.02	0.151±0.03	0.867±0.05	1690.81±30	15951.62
8	0.159±0.02	0.149±0.03	0.706±0.04	1306.43±30	13864.64
9	0.151±0.02	0.115±0.03	0.511±0.04	1246.53±30	10713.55
10	0.144±0.02	0.105±0.03	0.501±0.04	1201.13±30	9848.20

Values are the mean + S.D. of the 3 observations

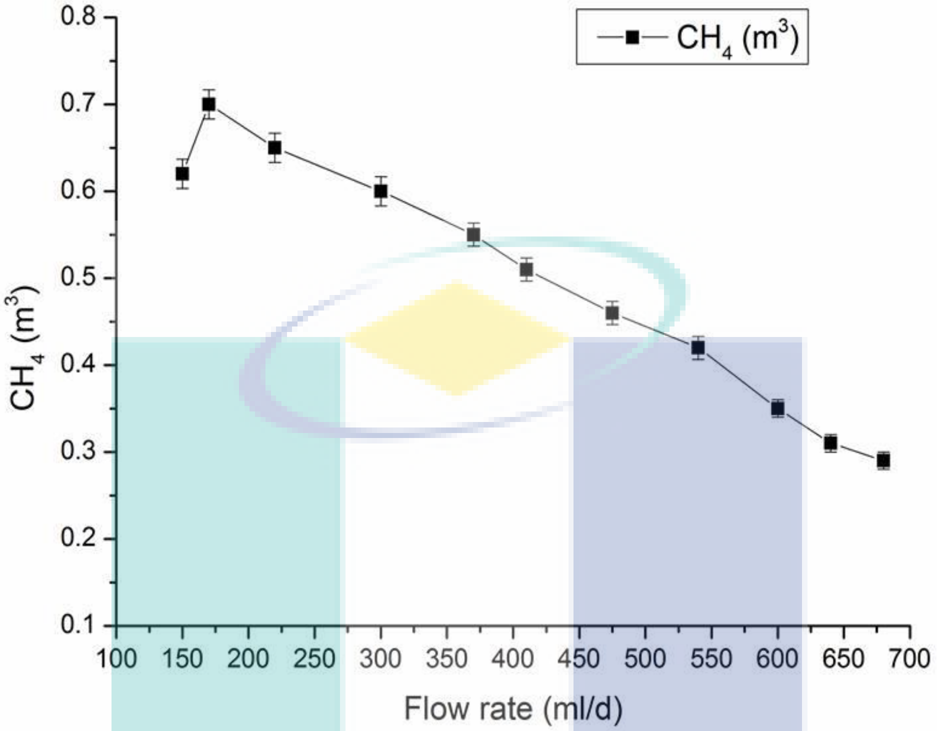

Figure: 4.16 Mean Methane production (m^3) from substrates at different flow rates (mL/day), where ■ -.CH_4 production.

4.17 BIOGAS GENERATION

The concept of co-digestion can be defined as the instantaneous digestion of a uniform blend of two or more wastes. Before AD, it is essential to perform a comprehensive characterisation of the wastewaters that will be subjected to treatment because their unique physicochemical properties strongly influence the outcome of the AD. The operational design strategy for the run of CSTR has been shown in Table 4.18. Hence, the results for the essential parameters of PWW, AM and co-digestion wastes (50:50), presented as the averages of the samples in triplicate, under mesophilic (37 ºC) conditions are presented in Table 4.18. The cumulative biogas yields (mL/g VS) from PWW and AW co-digestion at different F/M ratios (0.5, 1, 1.5 and 2) under mesophilic

(37 ºC) conditions are presented in Figure 4.17. Biogas generation started instantaneously on the initial day of co-digestion for all of the different F/M ratios (0.5, 1, 1.5 and 2). In particular, a gradual increase in cumulative biogas yields (mL/g VS) was observed up to 75 days of co-digestion for all of the F/M ratios. At the co-digestion period of 75 days, the values of cumulative biogas yields were 340 mL/g VS, 330 mL/g VS, 310 mL/g VS and 285 mL/g VS for the different F/M ratios of 0.5, 1, 1.5 and 2, respectively. Subsequently, a sharp increase in the values of cumulative biogas yields (mL/g VS) were observed a 105 day co-digestion period for the F/M ratio of 0.5. However, at this time, a steady increase in the values of cumulative biogas yields (mL/g VS) was observed for that of the F/M ratios of 1, 1.5 and 2. At the co-digestion period of 105 days, the values of the cumulative biogas yields were 599, 570, 550 and 530 mL/g VS for different F/M ratios of 0.5, 1, 1.5 and 2, respectively.

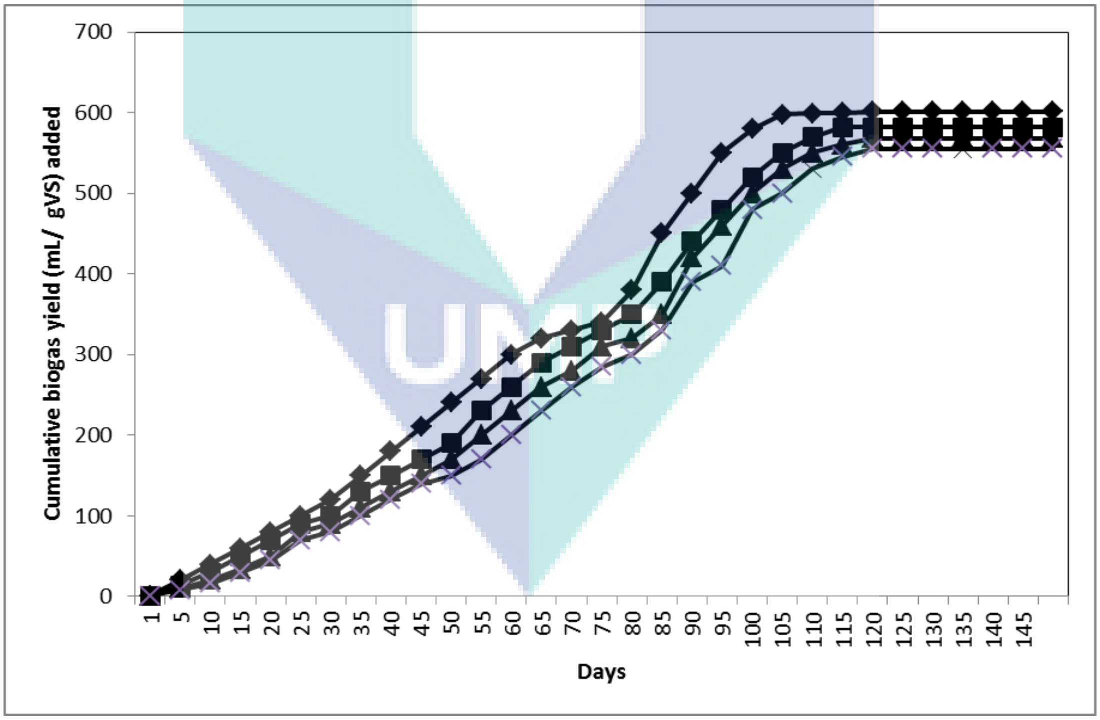

Figure 4.17 Cumulative biogas yield (mL/ g VS) from substrates at different F/M ratios and Mesophilic state (37 º C), where ♦-F/M 0.5, █ -F/M 1, ▲- F/M 1.5, **X-** F/M 2.

Thereafter, the values of cumulative biogas yields (mL/g VS) for all of the different F/M ratios (0.5, 1, 1.5 and 2) become steady. At the end of the co-digestion period of 145 days, the values of the cumulative biogas yields were 601, 582, 568 and 555 mL/g VS for the different F/M ratios of 0.5, 1, 1.5 and 2, respectively. Similarly, the cumulative biogas yields (mL/g VS) from PWW and AM co-digestion (50: 50) at different F/M ratios (0.5, 1, 1.5 and 2) under thermophilic (55 ºC) conditions are presented in Figure 4.18.

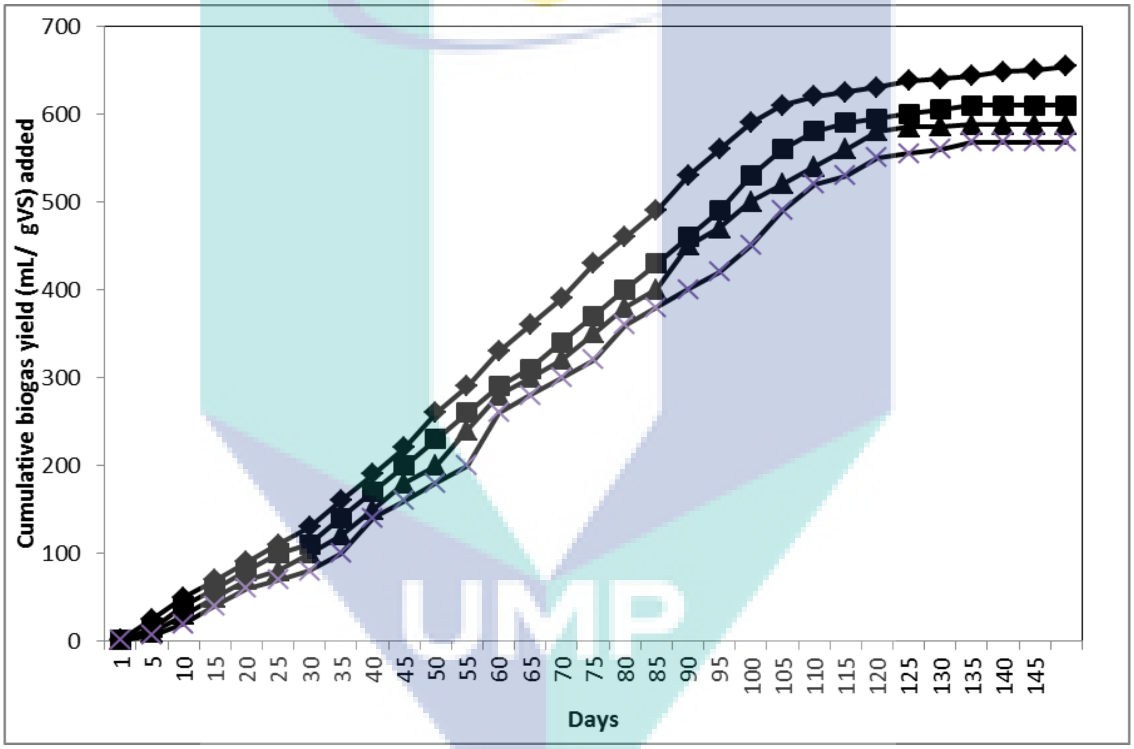

Figure 4.18 Cumulative biogas yield (mL/ g VS) from substrates at different F/M ratios and Thermophilic state (55 º C), where ♦-F/M 0.5, ■ -F/M 1, ▲- F/M 1.5, **X-** F/M 2.

At the end of 75 days of the thermophilic co-digestion period, the values of the cumulative biogas yields (mL/g VS) were observed to be noticeably higher than those of the mesophilic period for all of the F/M ratios. At this time, the values of the cumulative biogas yields were 430, 370, 350 and 320 mL/g VS for the different F/M ratios of 0.5, 1, 1.5 and 2, respectively. Subsequently, the cumulative biogas yield (mL/g VS) values were gradually increased up to 110 days. At the end of 110 days of the co-digestion period, the cumulative biogas yield (mL/g VS) values were 620, 580, 540 and 520 for the different F/M ratios of 0.5, 1, 1.5 and 2, respectively. Finally, the cumulative biogas generation becomes steady at the end of 145 days of the co-digestion period. The peak values at this state were 654, 610, 588 and 568 mL/g VS for the different F/M ratios of 0.5, 1, 1.5 and 2, respectively. Note that the final values of thermophilic cumulative biogas generation are higher when compared with that of mesophilic.

Table 4.18 CSTR operational outcomes at different F/M ratios and Mesophilic (37º C) state

	Units	Trial 1							
F/M ratio		0.25	0.5	0.75	1	1.25	1.5	1.75	2
Primary pH		6.1±0.02	6.11±0.02	6.13±0.01	6.15±0.02	6.18±0.02	6.23±0.02	6.28±0.02	6.32±0.02
Final pH		6.81±0.01	6.62±0.02	6.53±0.02	6.5±0.02	6.45±0.02	6.3±0.02	6.21±0.02	6±0.02
Biogas generation	mL/ gVS	571±12	601±15	590±14	582±14	575±13	568±13	560±12	555±11
Methane content	%	79.15±1.0	76.8±1.0	71.16±1.0	67.12±0.9	65.15±0.9	62.17±0.8	58.5±0.7	54.4±0.7
Methane generation	mL/ g VS	451.9±15	461.5±17	419.8±10	390.6±8	374.6±8	353.13±7	327.6±7	301.92±6
Removal of TS	%	43.13±0.2	45.5±0.2	46.12±0.3	49.2±0.3	50.31±0.3	51.15±0.4	52.16±0.4	53.18±0.4
Removal of VS	%	55.14±0.9	57.25±1	58.56±1	61.14±1.2	62.53±1.4	66.32±1.8	72.43±2.1	77.13±2.3
Biogas removed	g/gVS added	0.625±0.012	0.667±0.012	0.582±0.067	0.565±0.068	0.648±0.056	0.695±0.045	0.753±0.042	0.804±0.039
	g/gTS added	0.389±0.006	0.413±0.008	0.394±0.041	0.352±0.043	0.418±0.041	0.436±0.032	0.467±0.025	0.503±0.022
R^2		0.86	0.95	0.86	0.87	0.87	0.88	0.88	0.87

Table 4.19 CSTR operational outcomes at different F/M ratios and Thermophilic (55º C) state

	Units	**Trial 2**							
F/M ratio		0.25	0.5	0.75	1	1.25	1.5	1.75	2
Primary pH		6.1±0.02	6.12±0.02	6.15±0.01	6.18±0.02	6.22±0.02	6.3±0.02	6.4±0.02	6.45±0.02
Final pH		7.1±0.01	7.2±0.02	7.3±0.02	6.79±0.02	6.55±0.02	6.4±0.02	6.3±0.02	6.1±0.02
Biogas generation	mL/ gVS	620±12	654±15	622±12	610±11	599±11	588±10	575±10	568±9
Methane content	%	83.85±1.0	79.66±1.0	75.86±1.0	71.72±0.9	68.95±1.0	64.87±1.1	61.75±1.1	56.84±1.2
Methane generation	mL/ gVS	519.8±15	520.9±16	471.8±13	437.5±12	413±11	381.4±10	355±9	322.8±8
Removal of TS	%	34.13±1.2	36.8±1.6	39.12±1.3	43.2±3.3	44.31±3.2	44.75±3.2	45.16±1.1	46.18±1.1
Removal of VS	%	62.14±2.1	64.25±2.2	66.56±2.1	68.14±5.2	70.53±2.4	73.32±2.4	76.43±2.3	79.13±2.3
Biogas removed	g/g VS added	0.635±0.012	0.677±0.012	0.592±0.067	0.575±0.068	0.658±0.056	0.705±0.045	0.763±0.042	0.814±0.039
	g/g TS added	0.399±0.006	0.423±0.008	0.398±0.041	0.362±0.043	0.428±0.041	0.446±0.032	0.477±0.025	0.513±0.022
R^2		0.88	0.96	0.9	0.89	0.88	0.89	0.89	0.89

For the F/M ratios of 0.25, 1.25, 1.5 and 1.75, the mean biogas generations from the mesophilic CSTR were found to be 571, 590, 575 and 560 mL/g VS, respectively, and for the F/M ratios of 0.25, 1.25, 1.5 and 1.75, the mean biogas generations from thermophilic CSTR were 620, 622, 599 and 575 mL/g VS, respectively (Table 4.18 and 4.19). However, for both the thermophilic and mesophilic states, the biogas generation from PWW and AM co-digestion enhanced considerably as the F/M ratio increased from 0.25 to 0.5 but decreased gradually even the F/M ratio was increased up to 2. (Tables 4.18 and 4.19). It indicates that F/M ratio of 0.5 may be the optimum, as it provides the maximum biogas yield of 654±15 mL/g VS. A steady enhancement in biogas generation was studied by Zhu et al. (2014) when the F/M ratio was increased from 0.1 to 0.6. Moreover, the biogas generation remained constant for F/M 0.7 to 0.9. On the contrary, the biogas generation reduced considerably after 20 days of the digestion period of bean curd refuse-okra, once the F/M ratio surpassed 0.9.

4.18 METHANE GENERATION AND CONTENT

Figures 4.19 and 4.20 show the cumulative methane generation from experimental trials I and II. In particular, at the mesophilic (37 ºC) state, the cumulative methane generation increased steadily for all F/M ratios up to approximately the co-digestion period of 55 days (Figure. 4.19). At the co-digestion period of 75 days, the values of cumulative methane yields were 300, 280, 250 and 220 mL/g VS for the different F/M ratios of 0.5, 1, 1.5 and 2, respectively. Subsequently, a sharp increase in the values of the cumulative methane yields (mL/g VS) were observed up to 110 days of the co-digestion period for F/M ratios of 0.5. However, at this time, a steady increase in the values of the cumulative methane yields (mL/g VS) was observed for the F/M ratios of 1, 1.5 and 2. At the co-digestion period of 110 days, the values of cumulative methane yields were 420, 350, 335 and 297 mL/g VS for the different F/M ratios of 0.5, 1, 1.5 and 2, respectively. Thereafter, the values of the cumulative methane yields (mL/g VS) for all of the different F/M ratios (0.5, 1, 1.5 and 2) become steady.

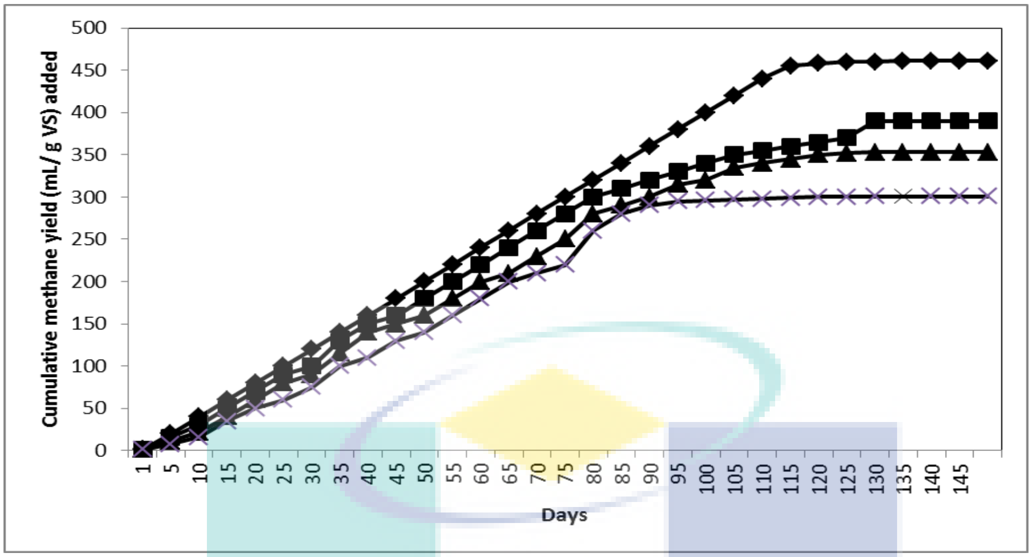

Figure 4.19 Cumulative methane yield (mL/ g VS) from substrates at different F/M ratios and Mesophilic state (37 º C), where ♦-F/M 0.5, █ -F/M 1, ▲- F/M 1.5, **X**- F/M 2.

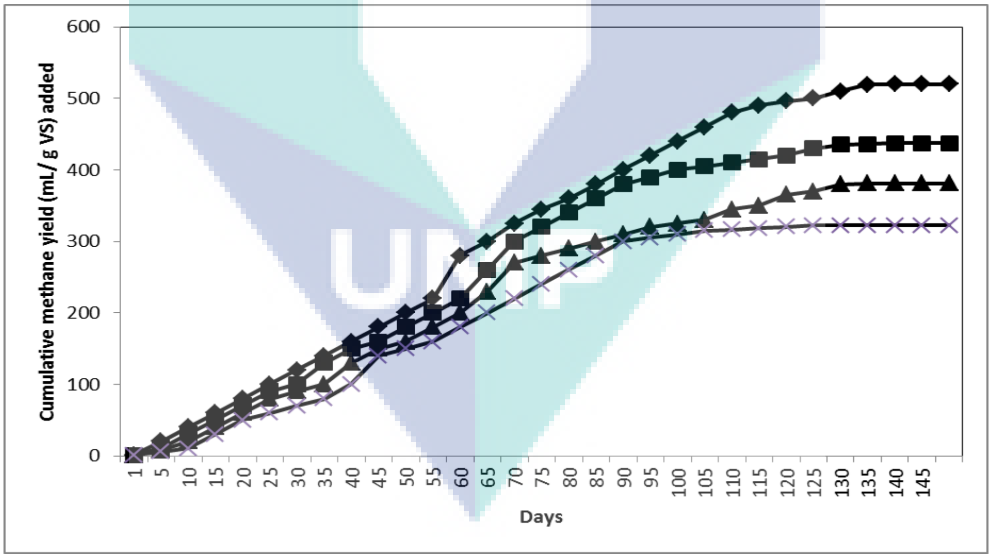

Figure 4.20 Cumulative methane yield (mL/ g VS) from substrates at different F/M ratios and Thermophilic state (55 º C), where ♦-F/M 0.5, █ -F/M 1, ▲- F/M 1.5, **X**- F/M 2.

At the end of the co-digestion period of 145 days, the values of cumulative biogas yields were 461, 390, 353 and 301 mL/g VS for the different F/M ratios of 0.5, 1, 1.5 and 2, respectively. Similarly, the cumulative methane yields (mL/g VS) from PWW and AW co-digestion (50:50) at the different F/M ratios (0.5, 1, 1.5 and 2) under thermophilic (55 ºC) conditions are presented in Figure. 4.18. At the end of 75 days of the thermophilic co-digestion period, the values of the cumulative methane yields (mL/g VS) were found to be noticeably higher than that of the mesophilic conditions for all of the F/M ratios. At this time, the values of the cumulative methane yields were 345, 320, 280, 240 mL/g VS. Subsequently, the cumulative biogas yield (mL/g VS) values were sharply increased up to 110 days. At the end of 110 days of the co-digestion period, the cumulative biogas yields (mL/g VS) values were 480, 410, 345 and 316 for the different F/M ratios of 0.5, 1, 1.5 and 2, respectively. Finally, the cumulative biogas generation becomes steady at the end of 145 days of the co-digestion period. The peak values at this state were 520, 437, 381 and 322 mL/g VS. It is notable that the final values of the thermophilic cumulative methane generation are higher when compared with that of the mesophilic case. However, it has been observed that the methane yield increased from 451.9±15 to 461.5±17 and 519.8±15 to 520.9±16 as the F/M ration increased from 0.25 to 0.5 for mesophilic and thermophilic states respectively but decreased gradually even the F/M ratio increased up to 2 (Table 4.18 and Table 4.19).

Figurer 4.21 showed that flow rate of 170 mL/day was significantly different from others. Therefore, F/M ratio of 0.5 may be considered as the optimum. Table 4.18 and Table 4.19 indicate that at the mesophilic state; the methane content (%) gradually decreased as the F/M ratios increased from 0.25 to 2. The mean methane percentage was observed to be 79.15%, 76.8%, 71.16%, 67.12%, 65.15%, 62.17%, 58.5% and 54.4% at F/M ratios of 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0, respectively, for the mesophilic state (Table 4.18) and 83.85%, 79.66%, 75.86%, 71.72%, 68.95, 64.87, 61.75% and 56.84% at F/M ratios of 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0, respectively, for the thermophilic state (Table 4.19). Note that the thermophilic methane content is approximately 3-4% higher than that of the mesophilic case. Zhang et al., 2013 observed 60% and 69% methane content in biogas from anaerobic co-digestion of both banana stem and swine manure for the mesophilic state and thermophilic state, respectively. However, for both conditions, the methane percentage of the biogas declined considerably as the F/M ratio increased from 0.25 to 2.0. Kolbl et al., 2014 observed an average methane generation of 250 mL/CH_4 g VS after 35 days of digestion from municipal wastewater at an F/M portion of 0.5 and for the mesophilic state. In addition, Kolbl et al., 2013 observed methane generation of 240 to 343 mL/CH_4 g VS at F/M ratios ranging from 0.5 to 1.5 (VS calculation) for plant substrate at the mesophilic state (37 ºC). Kawai et al., 2014 reported an average methane generation of 264 and 214 mL/CH_4 g VS from food waste at F/M ratios of 0.5 and 4 (VS calculation) for the mesophilic state (37 ºC). Silvestre et al., 2014 found an average methane generation of 354 and 512 mL/CH_4 g VS from grease waste at F/M ratios of 0.5 and 2 (VS calculation) for the thermophilic state (55 ºC).

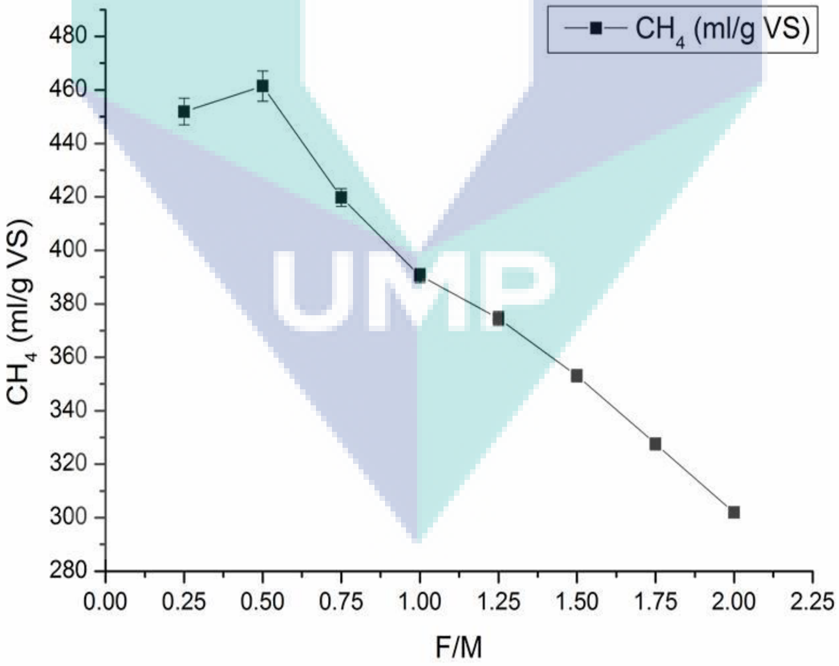

Figure 4.21 Mean methane yield (mL/ g VS) from substrates at different F/M ratios and Thermophilic state (37 ° C), where ■ -CH_4 production.

4.19 REMOVAL OF ORGANICS AND CHANGE OF pH

A significant increase in the TS and VS removal efficiencies can be observed from Tables 4.19 and 4.20 for both the mesophilic and thermophilic states as the F/M ratio gradually increased from 0.25 to 2. The TS removal efficiency was increased from 43.13% to 53.18% for the mesophilic state (Table 4.18) and 34.13% to 46.18% for the thermophilic state (Table 4.19) as the F/M ratio varied from 0.25-2. Similarly, the VS removal efficiency was from 55.14% to 77.13% for the mesophilic state (Table 4.18) and 62.14% to 79.13% for the thermophilic state (Table 4.19) as the F/M ratio varied from 0.25-2. Note that the thermophilic VS removal efficiency was considerably greater than that of the mesophilic case. The ultimate pH values for the mesophilic state were observed to be 6.8, 6.62, 6.53, 6.5, 6.45, 6.3, 6.21 and 6 at F/M ratios of 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0, respectively, and the ultimate pH values for the thermophilic state were observed to be 7.1, 7.2, 7.3, 6.79, 6.55, 6.4, 6.3 and 6.1 at F/M ratios of 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0, respectively (Table 4.18 and 4.19). A gradual drop was found in final pH values among the different F/M ratios (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0) and co-digestion conditions (37 and 55 ºC) with an OLR change from 6.31 to 27.14 g VS/L (Table 4.18), which caused system imbalance. A gradual enhancement in the biogas generation (mL/gVS) and the methane generation (mL/gVS) was found as the F/M ratio increased from 0.25 to 0.5 at both temperatures. This enhancement indicated that the PWW could simply be co-digested with AM without failure of the CSTR system, if F/M ratio of 0.5 is maintained. The regression value R^2 was maximum (0.96) for F/M ratio 0.5 for thermophilic condition. However, considering all the factors F/M ratio of 0.5 was observed to be the optimum to avoid the system imbalance.

4.20 RELATIONSHIPS AMONG BIOGAS, TS AND VS REMOVAL

The experimental biogas, TS and VS removal efficiency values are listed in Tables 4.18 and 4.19 or all of the operating conditions, the biogas removal efficiency was found to increase as the TS and VS removal efficiencies were increased. An excellent correlation was found to be observed between biogas removal and the TS and VS removal efficiencies when the CSTR was run at the thermophilic state compared to when it was run at the mesophilic state (Tables 4.18 and 4.19). Literature reports indicated that the biogas removal efficiency should be greater than that of the VS (Richards et al., 1991). In the present study, we observed the biogas removal efficiency to be greater than that of the VS. A highest difference of 22% was detected between the biogas removal efficiency and the VS removal efficiency when the F/M ratio was varied from 0.25- 2.0 for the mesophilic state; similarly, Kumar et al., 2014 observed the highest discrepancy of almost 23% from anaerobic degradation of textile wastewater.

4.21 CONCLUSIONS

This chapter represents the detail description of experimental output. The basic objectives have been explained with clear graphical presentation. The reactor performance has been discussed with adequate data in tubular forms. The comparative result reveals that the chemical and biological coupled treatment technology is highly accepted treating petrochemical wastewater. From the result section, it is clearly identified that anaerobic co-digestion strategy is capable enough to solve the burning problems occurring in commercial applications.

CHAPTER 5

CONCLUSIONS AND FUTURE WORKS

5.1 INTRODUCTION

This work has been addressed using chemical and biological coupled treatment technique to develop an anaerobic digestion process in CSTR. This episode précises the significant outcomes from the study performed. It also comprises several recommendations for future work in each of the areas covered during this research.

5.2 CONCLUSIONS

For the time being, CSTR is the most extensively practiced anaerobic reactor strategy treating vast range of wastewaters. Regardless waste treatment application and simplicity of installation makes it a unique technology. Despite such benefits of CSTR application is currently facing huge challenge to maintain desired degradation efficiency, process stability and methane yield. An obvious drawback of the existing CSTR is the lack of control over operating temperature and pH. This research investigates the combined effect of peroxidation pretreatment and anaerobic digestion strategy to overcome these challenges with significant enhancement in bioenergy production.

The addition of digital control of operating temperature and pH is an intensification of an existing CSTR. More specifically, chemical and biological coupled treatment technology (CBCTT) emphasizes on hydrolysis and methanogenesis. Oxidation by hydrogen peroxide (OHP) elevated the biodegradability of PWW along with COD reduction. Results revealed that 1% H_2O_2 dose (1.0mM Fe^{3+}) for 5 min exposure elevated biodegradability index (BOD_5/COD) up to 35%. Subsequently, anaerobic co-digestion of PWW with cattle manure (equal proportion) in CSTR provided the ultimate degradation efficiency.

Co-digestion of PWW with cattle manure in the equal proportion (50:50) successfully increased methane production up to 50-60% compared to conventional treatment systems. Mentionable that thermophilic condition (55°C) was proven to be the most suitable environmental condition to accelerate methanogenesis.

The various operational trials suggest that the optimum flow rate might be 170 mL/day, as it complies with the environmental guidelines; it provides the maximum digestion of the feed substrates. Additionally, such amount of energy may be utilized in a boiler to heat variety of liquids. Furthermore, it can also be used in an internal combustion engine installed to an electric generator to generate electrical energy. Consequently, pursuing a settlement between bio-digestion of the pilot plant, which complies existing legislature and a sensible size, which doesn't yield redundant installation cost, from all practices recognized that was being considered a retention time of 9 days. It is the novelty of this research, as the retention time of previous studies with this type of reactor are longer. As the PWW was digested almost completely by co-digesting with the AM, the final slurry may be expected to be used in the form of a soil improvement for reformative properties and performance equivalent to the available fertilizers in the shop. It indicates a novel conversion, but it may be essential for industries constructed in spaces without municipal wastewater networks.

The thermophilic (55 ºC) biogas and methane generation potential was detected to be significantly greater than that of the mesophilic (37 ºC). However, it has been observed that the methane yield increased from 451.9±15 to 461.5±17 and 519.8±15 to 520.9±16 as the F/M ratio increased from 0.25 to 0.5 for mesophilic and thermophilic states respectively but decreased gradually even the F/M ratio increased up to 2. Therefore, an F/M ratio of 0.5 might be the optimum. It is expected that the outcomes generated from this work might play an influential part for the design and implementation of industrial scale digester to treat PWW. A further research can be made for F/M proportions greater than 2 to Figure out the optimal range of F/M proportions for the biological treatment of PWW.

The sluggish growth of H_2 consuming methanogenic bacteria is responsible for Volatile fatty acid (VFA) accumulation. Inversely, the growth of glucose fermenting bacteria is fast enough producing H_2 accumulation. Frequently, it leads to reactor failure and sludge washes out. In this work, ammonium bicarbonate and co-digestion PWW with cattle manure were employed to avoid this problem. This strategy has proven its effectiveness by reducing VFA accumulation to a minimum of 100 mg/L. The system remained stable at OLR 6.31-27.24 kg COD/m^3 d with VFA/alkalinity ratio less than 0.4. It is obvious that operating temperature is a momentous factor during biodegradation. The ultimate COD degradation efficiency from the chemical and biological coupled treatment technology was achieved up to 98±0.5% at masophilic condition (37˚c), which indicates the environmental feasibility of this technology.

This research does provide the following novel contributions: The first major contribution of the research is the framework of chemical and biological coupled treatment technology (CBCTT). The second contribution is its successful implementation treating PWW along with the compliance to the environmental regulations as well.

The key findings of anaerobic co-digestion of PWW with AM are:

1. An Intensified CSTR reactor resulted in successful treatment of PWW with AM in equal proportion at 37^oC and 55^oC. The addition of digital control of operating temperature and pH and co-digestion of AM with PWW improved methane production compared to control and COD reduction. In this respect, this technology shows a promising option to improve the effluent quality.

2. Optimum flow rate was an important operating parameter that improved the COD degradation and methane production efficiency. Lower Influent flow rates (170 mL/day) improved bioconversion of PWW to methane.

3. Optimum F/M ratio was a key parameter that enhanced the COD degradation and methane production efficiency. Influent F/M ratio of 0.5 enhanced bioconversion of PWW to methane.

4. VFA accumulation and sludge wash out were the major problems of the reactor failure. Application of 10 mg/L ammonium bicarbonate and co-digestion PWW with cattle manure in equal proportion successfully reduced the VFA accumulation.

5.3 FUTURE WORK

The effect of chemical pretreatment and subsequent anaerobic co-digestion in CSTR performance treating PWW was investigated. The successful application of this new technology can be expanded by recommending further research below:

1. PWW that requires small area for the setup of treatment plant and energy recovery in terms of biogas should be investigated by CSTR reactor. Furthermore, operation costs can be reduced through utilization of biogas for

heat or electricity energy generation in the plant. This method is also able to treat effluent to a satisfactory quality for discharge at lower costs.

2. Further invetigations should be carried out for a longer duration to ascertain upper limits for higher organic loading rates.

3. Models are useful tools in the development and design of CSTR reactors. Significant model should be investigated for researchers to study the consequences for the reactor performance of different types of substrate, different inlet substrate concentration, different flow rates and different kinds of biomass.

4. The microbial activity of CSTR reactor in terms of biogas should be monitored for enhancing biogas production.

5. The CSTR reactor is presently successfully applied for wastewater containing non-inhibitory substrate at high concentration, e.g., distillery waste, brewery waste, and sugar industry waste. A suitable modification for the treatment of complex industrial wastewaters in the presence of some inhibitory compounds is a challenge. Development in this direction will lead to successful application of CSTR for milk and food processing waste, gelatine manufacturing plant waste, slaughterhouse waste, etc.

6. Another challenge is to shorten the start-up time of the reactor by enhancing granule formation. Various external additives have shown promising results in this direction; however, most of these studies are limited to laboratory scale reactor. The effect of these additives should be investigated in pilot-scale reactors along with the economics of the additives.

REFERENCES

Aboulhassan, M.A., Souabi, S. and Yaacoubi, A. 2008. Pollution reduction and biodegradability index improvement of tannery effluents. *International journal of Environmental Science and Tecnology*. **5** (1): 11-16.

Agustina, T.E. and Vareek, H.M. 2005. A review of synergistic effect of photocatalysis and ozonation on wastewater treatment. *Journal of Photochemistry and Photobiology*. **C**: 264-273.

Ahad, J.M.E. and Slater, G.F. 2008. Carbon isotope effects associated with Fenton-like degradation of toluene: potential for differentiation of abiotic and biotic degradation. *Science of The Total Environment*. **401**: 194–198.

Ahmad, A. L., Ismail, S., Ibrahim, N. and Bhatia, S. 2003. Removal of suspended solids and residual oil from palm oil mill effluent. *Journal of Chemical Technology and Biotechnology*. **78** (9): 971–978.

Ahmad, A., Ghufran, R. and Faizal, W.M. 2010. Cd(II), Pb(II) and Zn(II) removal from contaminated water by Biosorption using activated sludge biomass. *Clean*. **38**(2): 153-158.

Ahn, J.H. and Forster, C.F. 2002. A comparison of mesophilic and thermophilic anaerobic upflow filters treating paper-pulp-liquors. *Process Biochemistry*. **38**: 257–262.

Alatriste-Mondragon, F., Samar, P., Cox, H.H.J., Ahring, B.K. and Iranpour, R. 2006. Anaerobic codigestion of municipal, farm, and industrial organic wastes: a survey of recent literature. *Water Environment Research*. **78**: 607–636.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K. and Walter, P. 2002. *Peroxisomes*. 4th ed. New York: Garland Science.

Alvarez, J. M., Dosta, J., Güiza, M.S.R, Fonoll, X., Peces, M. and Astals, S. 2014. A critical review on anaerobic co-digestion achievements between 2010 and 2013. *Renewable and Sustainable Energy Reviews*, **36**: 412-427.

Angelidaki, I. and Ahring, B. K. 2000. Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. *Water Science and Technology*. **41**: 189–194.

Angelidaki, I., Chen, X., Cui, J., Kaparaju, P. and Ellegaard, L. 2006. Thermophilic anaerobic digestion of source-sorted organic fraction of household municipal solid waste: Start-up procedure for continuously stirred tank reactor. *Water Research.* **40**: 2621-2628.

Angenent, L.T., Karim, K., Al-dahhan, M.H., Wrenn, B.A. and Espinosa, R.D. 2004. Production of bioenergy and biochemical from industrial and agricultural wastewater. *TRENDS in Biotechnology*. **22** (9): 477- 485.

Aoki, N. and Kawase, M. 1991. Development of high performance thermophilic two phase digestion process. *Water Science and Technology*. **23**: 1147–1156.

APHA. 2012. Standard methods for the examination of water and wastewater, 22 th ed. Am Public Health Association, Washington, D.C.

Apollo, S., Onyango, M.S. and Ochieng, A. 2014. Integrated UV photodegradation and anaerobic digestion of textile dye for efficient biogas production using zeolite. *Chemical Engineering Journal*. **245**: 241-247.

Ayati, B. and Ganjidoust, H. 2006. Comparing the efficiency of UAFF and UASB with hybrid reactor in treating wood fiber wastewater. *Iranian Journal of Environmental Health Science Engineering.* **3**: 39–44.

Babu, B.R., Meera, K.S., Venkatesan, P. and Sunandha, D. 2010. Removal of Fatty acids from Palm Oil Effluent by Combined Electro-Fenton and Biological Oxidation process. *Water Air Soil pollution.* **211**: 203-210.

Bardiya, N. and Gaur, A.C. 1997. Effects of carbon and nitrogen ratio on rice straw bio-methanation. *Journal of Rural Energy*. **4** (1–4): 1–16.

Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhnyi, S.V., Pavlostathis, S.G., Rozzi, A., Sanders, W.T.M., Siegrist, H. and Vavilin, V. 2002. *Anaerobic digestion models no 1 (ADM1).* UK: IWA Publishing.

BBC, 2007. *Gore and UN panel win Nobel Prize*. London: BBC World News.

Bendixen, H.J. 1994. Safeguards against pathogens in Danish biogas plants. *Water Science and Technology*. **30**: 171–180.

Bierkens, J. and Geerts L. 2014. Environmental hazard and risk characterisation of petroleum substances: A guided “walking tour” of petroleum hydrocarbons. *Environment International.* **66**:182-193.

Bok, D., Plugge C.M. and Stams, A.J.M. 2004. Interspecies electron transfer in methanogenic propionate degrading consortia. *Water Research.* **38**: 1368-1375.

Borja, R. and Banks, C.J. 1994b. Treatment of palm oil mill effluent by upflow anaerobic filtration. *Journal of Chemical Technology and Biotechnology*. **61**: 103–109.

Borowski, S., Domański, J. and Weatherley, L. 2014. Anaerobic co-digestion of swine and poultry manure with municipal sewage sludge. *Waste Management*. **34**: 513-521.

Bouallagui, H., Rachdi, B., Gannoun, H. and Hamdi, M. 2009b. Mesophilic and thermophilic anaerobic co-digestion of abattoir wastewater and fruit and vegetable waste in anaerobic sequencing batch reactors. *Biodegradation.* **20**: 401–409.

Braun, R. 2002. Potential of Co-digestion (On line). http:// www.iea-biogas.net/_.../...(Cited on April 14, 2012).

Brehmera, B., Boomb, R.M. and Sanders, J. 2009. Maximum fossil fuel feedstock replacement potential of petrochemicals via biorefineries. *Chemical Engineering Research and Design.* **87**: 1103–1119.

Britz, T.J., Meyer, L.C. and Botes, P.J. 1983. Anaerobic digestion of a petrochemical effluent. *Biotechnology letters.* **5** (2): 115-118.

Bustamante M.A., Restrepo A.P., Alburquerque J.A., Pérez-Murcia M.D., Paredes C., Moral R. and Bernal M.P., 2013. Recycling of anaerobic digestates by composting: effect of the bulking agent used. *Journal of Cleaner Production.* **47**: 61-69.

Cao,Y. and Pawłowski, A. 2012. Sewage sludge-to-energy approaches based on anaerobic digestion and pyrolysis: Brief overview and energy efficiency assessment. *Renewable and Sustainable Energy Reviews.* **16**: 1657-1665.

Callaghan, F.J., Wase, D.A.J., Thayanithy, K. and Forster, C.F. 2002. Continuous co digestion of cattle slurry with fruit and vegetable wastes and chicken manure. *Biomass Bioenergy.* **22** (1): 71–7.

Chaillan, F., Flecheb, A., Burya, E., Phantavonga, Y., Saliot, A. and Oudot, J. 2004. Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms. *Research in Microbiology.* **155** (7): 587-595.

Chamarro, E., Marco, A. and Esplugas, S. 2001. Use of Fenton reagent to improve organic chemical biodegradability. *Water Research.* **35**: 1047.

Chan, K.S. and Chooi, C.F. 1984. Ponding System for palm oil mill effluent treatment. *In: Proceedings of the Regional Workshop on Palm Oil Mill Technology & Effluent Treatment*. pp. 185–192.

Chance, B., Sies, H. and Boveris, A. 1979. Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* **59**: 527-605.

Chandra, R., Vijay, V.K., Subbarao, P.M.V. and Khura, T.K. 2012. Production of methane from anaerobic digestion of jatropha and pongamia oil cakes. *Applied Energy*. **93**: 148–159.

Chandra, R. and Mohan S.V. 2014. Enhanced bio-hydrogenesis by co-culturing photosynthetic bacteria with acidogenic process: Augmented dark-photo fermentative hybrid system to regulate volatile fatty acid inhibition. *International Journal of Hydrogen Energy*. **39**: 7604-7615.

Chelikani, P., Fita, I. and Loewen, P.C. 2004. Diversity of structures and properties among catalases. *Cellular and Molecular Life Sciences.* **61** (2): 192–208.

Chen, Y., Cheng, J.J. and Creamer, K.S. 2008. Inhibition of anaerobic digestion process: A review. *Bioresource Technology*. **99**: 4044-4064.

Chen and Pan,1996. Assay of superoxide dismutase activity. *Bot. Bull. Acad. Sin.* **37**: 107-111.

Conivas-Diaz, M. and Howell, J.A. 1988. Down-flow foxed film anaerobic reactor stability studies under organic and Hydraulic overloading at different working volumes. *Water Research.* **22**: 529-538.

Cord-Ruw-Isch, R. and Mercz, T.I. 1996. Dissolved hydrogen concentration as an online control parameter for the automated operation and optimization of anaerobic digesters. *Biotechnology and Bioengineering.* **56** : 626-634.

Dareioti, M.A. and Kornaros, M. 2014. Effect of hydraulic retention time (HRT) on the anaerobic co-digestion of agro-industrial wastes in a two-stage CSTR system. *Bioresource Technology*. **167**: 407-415.

Dhar, B.R., Nakhla, G. and Ray, M.B. 2012. Techno-economic evaluation of ultrasound and thermal pretreatments for enhanced anaerobic digestion of municipal waste activated sludge. *Waste Management.* **32**: 542–549.

Diana M. Arroyave G., Darío Gallego S. and Marco A. Márquez G. 2013. Evaluation of residence time distribution and mineralogical characterization of the biooxidation of sulfide minerals in a continuous stirred tank reactor. *Minerals Engineering*, **46–47**: 128-135.

Dsikowitzky, L., Schwarzbauer, J. and Littke, R. 2004b. The anthropogenic contribution to the organic load of the Lippe River (Germany). Part II: quantification of specific organic contaminants. *Chemosphere*. **57**: 1275–1288.

Dsikowitzky, L., Schwarzbauer, J., Kronimus, A. and Littke, R., 2004a. The anthropogenic contribution to the organic load of the Lippe River (Germany). Part I: qualitative characterization of low-molecular weight organic compounds. *Chemosphere*. **57**: 1275–1288.

Eng, S.C. and Fernandez, X.A. 1986. Biochemical effects of administering shock loads of sucrose to laboratory UASB effluent treatment plant. *Water Research*. **20** (6): 784-789.

Environmental Quality Act (Prescribed Premises) Order 1974 (On line). www.doe.gov.my/portal/wp-content/.../A-Guide-For-Investors1.pdf (4 March 2012).

Environmental Quality Act (Scheduled Wastes) Regulations 2009. www.doe.gov.my/portal/wp-content/.../A-Guide-For-Investors1.pdf (4 March 2012).

Fang, H.H.P. and Chung, D.W.C. 1999. Anaerobic treatment of proteinaceous wastewater under mesophilic and thermophilic conditions. *Water Science and Technology*. **40**: 77–84.

Ferguson, S. H., Woinarski, A.Z., Snape, I., Morris, C.E. and Revill, A.T. 2004. A field trial of in situ chemical oxidation to remediate long-term diesel contaminated Antarctic soil. *Cold Regions Science and Technology*. **40**: 47–60.

Ferrer, P., López, M.C., Cerisuelo, A., Peñaranda, D.S. and Moset, V. 2014. The use of agricultural substrates to improve methane yield in anaerobic co-digestion with pig slurry: Effect of substrate type and inclusion level. *Waste Management*. **34**: 196-203.

Fulford, D. 1988. *Running a Biogas Programme: A handbook*. The Biogas Technology in China: IT Publications No. 52.

Gaetani, G., Ferraris, A., Rolfo, M., Mangerini, R., Arena, S. and Kirkman, H. 1996. Predominant role of catalase in the disposal of hydrogen peroxide within human erythrocytes. *Blood*. **87** (4): 1595–9.

Gaetani, G.F., Galiano, S., Canepa, L., Ferrris, A.M. and Kirkman, H.N. 1989. Catalase and glutathione peroxidase are equally active in detoxification of Hydrogen peroxide in human erythrocytes. *Blood*. **73**: 334-339.

Gasim, H.A., Kutty, S.R.M. and Isa, M.H. 2010. Biodegradability of Petroleum Refinery Wastewater in Batch Reactor. *Working paper. International Conference on Sustainable Building and Infrastructure (ICSBI'10),* World Engineering, Science and Technology Congress (ESTCON). KLCC, Malaysia: 15-17 June.

Gelegenis, J., Georgakakis, D., Angelidaki, I., Christopoulou, N. and Goumenaki, M. 2007. Optimization of biogas production from olive-oil mill wastewater by co-digesting with diluted poultry-manure. *Applied Energy*. **84** (6): 646–663.

Gerardi, M.H. 2003. *The microbiology of anaerobic digesters*. USA: Wiley-Interscience. 103-108.

Ghorbanian M., Moussavi G. and Farzadkia M. 2014. Investigating the performance of an up-flow anoxic fixed-bed bioreactor and a sequencing anoxic batch reactor for the biodegradation of hydrocarbons in petroleum-contaminated saline water. *International Biodeterioration & Biodegradation*. **90**: 106-114.

Ghosh, D., Sagar, A.D. and Kishore, V.V.N. 2006. Scaling up of Biomass Gasifier Use: An Application-specific Approach. *Energy Policy*. **34**: 1566-1582.

Goberna, M., Schoen, M. A., Sperl, D., Wett, B. and Insam, H. 2010. Mesophilic and thermophilic co-fermentation of cattle excreta and olive mill wastes in pilot anaerobic digesters. *Biomas. Bioeng.* **34** (3): 340–346.

Goodsell, D.S. 2004. *Catalase. Molecule of the Month*. RCSB Protein Data Bank. Retrieved 2007-02-11.

Goodwin, J.A.S., Wase, D.A.J. and Forster, C.F. 1992. Pre-granulated seeds for UASB reactors: how necessary are they? *Bioresource Technology*. **41**: 71–79.

Guijer, W. and Zhender, A.J.B. 1983. Convirsion process in anaerobic digestion. *Water Science and Technology*. **15** :127-167.

Guo, L., Shi, Y., Zhang, P., Wu, L., Gai, G.S., Wang, H. and Xiao, D.L. 2011. Investigations, analysis and study on biogas utilization in cold region of North China. *Advanced Material Research.* **183–185**: 673–677.

Gupta, P., Singh, R.S., Sachan, A., Vidyarthi, A.S. and Gupta, A. 2012. Study on biogas production by anaerobic digestion of garden-waste. *Fuel.* **95**: 495–498.

Guwy, A.J., Martin, M.H., Hawkes, F.R. and Hawkes, D.L. 1999. Catalase activity measurements in suspended aerobic biomass and soil samples. *Enzyme and Microbial Technology*. **25**: 669–676.

Hamdi, M. and Garcia, J.L. 1991. Comparison between anaerobic filter and anaerobic contact process for fermented olive mill wastewaters. *Bioresource Technology.* **38**: 23–29.

Hao, X., Heijnen, J.J. and Loosdrecht, M.C.M. 2002. Sensitivity analysis of a biofilm model describing a one-stage completely autotrophic nitrogen removal (CANON) process. *Biotechnology and Bioengineering*. **77** (3): 266–277.

Harsono, S.S., Grundmann, P. and Soebronto, S. 2014. Anaerobic treatment of palm oil mill effluents: potential contribution to net energy yield and reduction of greenhouse gas emissions from biodiesel production. *Journal of Cleaner Production*. **64**: 619-627.

Hartmann, H. and Ahring, B.K. 2005. Anaerobic digestion of the organic fraction of municipal solid waste: influence of co-digestion with manure. *Water Research.* **39**: 1543–1552.

Hashimoto, A.G., Varel, V.H. and Chen, Y.R. 1981. Ultimate methane yield from beefcattle manure – effect of temperature, ration constituents, antibiotics and manure age. *Agricultural Wastes.* **3**: 241–256.

Hegde, G. and Pullammanappallil, P. 2007. Comparison of thermophilic and mesophilic one-stage, batch, high-solids anaerobic digestion. *Environmental. Technology*. **28**: 361–369.

Hidalgo, D., and Marroquín J.M.M. 2014. Effects of inoculum source and co-digestion strategies on anaerobic digestion of residues generated in the treatment of waste vegetable oils. *Journal of Environmental Management*. **142**: 17-22.

Hua, Z., Yue, Z., Yu, H., Liu, S., Harada, H. and Li, Y. 2012. Mechanisms of microwave irradiation pretreatment for enhancing anaerobic digestion of cattail by rumen microorganisms. *Applied Energy*. **93**: 229–236.

Hwang, B.K., Son, H.S., Kim, J.H., Ahn, C.H., Lee, C.H., Song, J.Y. and Ra, Y.H. 2010. Decomposition of excess sludge in a membrane bioreactor using a turbulent jet flow ozone contactor.*Journal of Industrial and Engineering Chemistry*. **16**: 602-608.

IEA Bioenergy, 2009. Bioenergy - A Sustainable and Reliable Energy Source (Online). http://www.ieabioenergy.com/ (4 May 2012).

Ike, M., Inoue, D., Miyano, T., Liu, T.T., Sei, K., Soda, S. and Kadoshin, S. 2010. Microbial population dynamics during startup of a full-scale anaerobic digester treating industrial food waste in Kyoto eco-energy project. *Bioresource Technology*. **101**: 3952–3957.

ITRC. 2001. *Technical and Regulatory Guidance for In Situ Chemical Oxidation of Contaminated Soil and Groundwater.* Washington, DC: Interstate Technology and Regulatory Council.

ITRC. 2005. *Technical and Regulatory Guidance for In Situ Chemical Oxidation of Contaminated Soil and Groundwater.* Washington, DC: Interstate Technology and Regulatory Council.

Jafarzadeh, M.T., Mehrdadi, N., Azimi, A. A. and Hashemian, S. J. 2006. Petrochemical waste water treatment using anaerobic hybrid reactor. *Pakistan journal of Biological Sciences*. **9** (6): 1037-1042.

Jennifer, G.A., Marc, W.B., Doglas, R.C. and Allison, M.F. 2010. Effects of oxygenation on ammonia oxidation potential and microbial diversity in sediment from surface-flow wetland mesocosms. *Bioresource Technoogyl*. **101** (4): 1389-1392.

Jeong, E., Kim, H., Nam, J. and Shin, H. 2010. Enhancement of bioenergy production and effluent quality by integrating optimized acidification with submerged anaerobic membrane bioreactor. *Bioresource Technology*. **101**: 1873–2976.

Kafle, G.K. and Kim, S.H., 2013a. Effects of chemical compositions and ensiling on the biogas productivity and degradation rates of agricultural and food processingby-products. *Bioresource Technology*. **142**: 553-561.

Kalyuzhnyi, S., de- los- Santos, L.E. and Martinez, J.R. 1998. Anaerobic treatment of raw and preclarified potato-maize wastewater in a UASB reactor. *Bioresource Technology*. **66**: 198–199.

Kaparaju P., Luostarinen S., Kalmari E., Kalmari J., Rintala J., 2000. Co-digestion of energy crops and industrial confectionery by-products with cow manure: batch-scale and farm-scale evaluation. *Water Science and Technology*. **41**: 189–194.

Kaparaju, P., Buendia, I., Ellegaard, L. and Angelidakia, I. 2008. Effects of mixingon methane production during thermophilic anaerobic digestion of manure: lab-scale and pilot-scale studies. *Bioresource Technology*. **99**: 4919– 4928.

Karim, K., Hoffmann, R., Klasson, K.T. and Al-Dahhan, M.H. 2005b. Anaerobic digestion of animal waste: effect of mode of mixing. *Water Research*. **39**: 3597–3606.

Karim, K., Hoffmann, R., Klasson, T. and Al-Dahhan, M.H. 2005c. Anaerobic digestion of animal waste: waste strength versus impact of mixing. *Bioresource Technology*. **96**: 1771–1781.

Karim, K., Klasson, K.T., Hoffmann, R., Drescher, S.R., DePaoli, D.W. and Al-Dahhan, M.H. 2005a. Anaerobic digestion of animal waste: effect of mixing. *Bioresource Technology*. **96**: 1607–1612.

Kaspar, H. and Wuhrman, K. 1978. Product inhibition in sludge digestion. *Microbiol. Ecol.* **4**: 241-248.

Kawai, M., Nagao, N., Tajima, N., Niwa, C., Matsuyama, T. and Toda, T. 2014. The Effect of the Labile Organic Fraction in Food Waste and the Substrate/Inoculum ratio on Anaerobic Digestion for a Reliable Methane Yield. *Bioresource Technology*, In Press, Accepted Manuscript, Available online 30 January 2014.

Kennedy, K.J. and Vanden Berg, L. 1982. Stability and performance of anaerobic fixed film reactors during hydraulic overloading at 10 to 35 ° C. *Water Research.* **16**: 1391-1398.

Khaing, T.H., Li, J., Li, Y., Wai, N. and Wong, F.S. 2010. Feasibility study on petrochemical wastewater treatment and reuse using a novel submerged membrane distillation bioreactor. *Separation and Purification Technology.* **74**: 138–143.

Khan, E., Wirojanagud, W. and Sermsai, N. 2009. Effects of iron type in Fenton reaction on mineralization and biodegradability enhancement of hazardous organic compounds. *Journal of Hazardous Materials.* **161**: 1024 – 1034.

Kim, I. and Tanaka, H. 2010. Use of ozone-based process for the removal of pharmaceuticals detected in a wastewater treatment plant. *Water Environment Research.* **82** (4): 294-301.

Kim, M., Ahn, Y.H. and Speece, R.E. 2002. Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic. *Water Research.* **36**: 4369–4385.

Kirchman, D.L., Suzuki, Y., Garside, C. and Ducklow, H.W. 1991. High turnover rates of dissolved organic carbon during a spring phytoplankton bloom. *Nature.* **352**: 612 – 614.

Kizilkaya, R. and Bayrakli, B. 2005. Effects of N-enriched sewage sludge on soil enzyme activities. *Applied Soil Ecology*. **30**: 192–202.

Kleinstreuer, C. and Poweigha, T. 1982. Dynamic simulator for anaerobic digestion process. *Biotechnology and Bioengineering.* **24**: 1941-1951.

Kolbl, S., Jerman, V., Novak, D., Bozic, J. and Stres, B., 2013. Bacterial and archaeal microbial community dynamics during the start-up of 4 MW biogas plant Keter Organica Nova. H. Insam, S. Podmirseg, A.O. Wagner (Eds.), 8th International Symposium on Anaerobic Microbiology (ISAM8), Innsbruck, Austria, June 12–15, 2013, (Conference Series) (first ed.), Innsbruck University Press, Innsbruck, pp. 105.

Kolbl, S., Paloczi, A., Panjan, J. and Stres, B., 2014. Addressing case specific biogas plant tasks: Industry oriented methane yields derived from 5 L Automatic Methane Potential Test Systems in batch or semi-continuous tests using realistic inocula, substrate particle sizes and organic loading. *Bioresource Technology*. **153**: 180-188.

Kong, S. H., Watts, R.J. and Choi, J.H. 1998. Treatment of petroleum-contaminated soils using iron mineral catalyzed hydrogen peroxide. *Chemosphere.* **37** (8): 1473–1482.

Kosaka, T., Uchiyama, T., Ishii, S., Enoki, M., Imachi, H., Kamagata, Y., Ohashi, A., Harada, H., Ikenaga, H. and Watanabe, K. 2006. Reconstruction and regulation of the central catabolic pathway in the thermophilic propionate-oxidizing syntroph Pelotomaculum thermopropionicum. *Journal of Bacteriology*. **188**: 202–210.

Kozmér, Z., Arany, E., Alapi, T., Takács, E., Wojnárovits, L. and Dombi, A. 2014. Determination of the rate constant of hydroperoxyl radical reaction with phenol. *Radiation Physics and Chemistry*. **102**:135-138.

Kumar, K., Singh G.K., Dastidar, M.G., Sreekrishnan, T.R., 2014. Effect of mixed liquor volatile suspended solids (MLVSS) and hydraulic retention time (HRT) on the performance of activated sludge process during the biotreatment of real textile wastewater. *Water Resources and Industry*, In Press, Accepted Manuscript, Available online 21 January 2014.

Kurimoto, N., Brumfield, B., Yang, X., Wada, T., Diévart, P., Wysocki, G., and Ju Y. 2014. Quantitative measurements of HO_2/H_2O_2 and intermediate species in low and intermediate temperature oxidation of dimethyl ether. *Proceedings of the Combustion Institute*, In Press, Corrected Proof, Available online 21 June 2014.

Kythreotou, N., Florides, G. and Tassou S.A. 2014. A review of simple to scientific models for anaerobic digestion. *Renewable Energy*. **71**: 701-714.

Lahavand, O. and Morgan, B. 2004. Titration methodologies for monitoring of anaerobic digestion in developing countries—a review. *Journal of Chemical Technology and Biotechnology*. **79** :1331–1341.

Lakatos, G., Veres, Z., Kundrát, J. and Ilona Mészáros, I. 2014. The management and development of constructed wetlands for treatment of petrochemical waste waters in Hungary: 35 years of experience. *Ecohydrology & Hydrobiology*. **14**: 83-88.

Latif, M.A., Ghufran, R., Zularisam, A.W. and Ahmad, A. 2011. Integrated application of upflow anaerobic sludge blanket reactor for the treatment of wastewaters. *Water Research*, **45**: 4683-4699.

Lee, J.C., Kim, J.H., Chang, W.S. and Pak, D. 2012. Biological conversion of CO_2 to CH_4 using hydrogenotrophic methanogen in a fixed bed reactor. *J Chem Technol Biotechnol.* DOI: 10.1002/jctb.3787.

Leslie, G.J.R., Daigger, G.T. and Lim, H.C. 1999. *Biological Wastewater Treatment.* 2nd ed. Essex: CRC Press. Revised & Expanded.

Lettinga, G. 1995. Anaerobic digestion and wastewater treatment systems. *Antonie van Leeuwenhoek*. **67**: 3–28.

Li, W., Nanaboina, V., Zhou,Q. and Korshin, G.V. 2013. Changes of excitation/emission matrixes of wastewater caused by Fenton- and Fenton-like treatment and their associations with the generation of hydroxyl radicals, oxidation of effluent organic matter and degradation of trace-level organic pollutants. *Journal of Hazardous Material*. **244–245**: 698-708.

Lindmark, J., Eriksson, P. and Thorin E. 2014. The effects of different mixing intensities during anaerobic digestion of the organic fraction of municipal solid waste. *Waste Management*. **34**: 1391-1397.

Liu, X., Gao, X., Wang, W., Zheng, L., Zhou, Y. and Sun, Y. 2012. Pilot-scale anaerobic co-digestion of municipal biomass waste: Focusing on biogas production and GHG reduction. *Renewable Energy*. **44**: 463-468.

Long, T., Anas, A., Zhai, J. and Lin, M.B. 2007. The effect and its influence factors of the fenton process on the old landfill leachate. *Journal of Applied Sciences*. **7** (5): 724-727.

Lu, F., Hao, L., Guan, D., Qi, Y., Shao, L. and He, P., 2013. Synergetic stress of acids and ammonium on the shift in the methanogenic pathways during thermophilic anaerobic digestion of organics. Water Research. **47**, 2297-2306.

Lu, J., Li, D., Chen, L., Kitamura, Y., Jiang, W. and Li, B. 2014. Simultaneous pretreatment and acidogenesis of solid food wastes by a rotational drum fermentation system with methanogenic leachate recirculation and andesite porphyry addition. *Bioresource Technology*. **138**: 101-108.

Lund, B., Jensen, V.F., Have, P. and Ahring, B. 1996. Inactivation of virus during anaerobic digestion of manure in laboratory scale biogas reactors. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*. **69**: 25–31.

Luo, G., Xie, L., Zhou, Q. and Angelidaki, I. 2011. Enhancement of bioenergy production from organic wastes by two-stage anaerobic hydrogen and methane production process. *Bioresource Technology*. doi: 10.1016/j.biortech. 2011.02.012.

Lynd, L.R., Weimer, P.J., Zyl, W.H. and Pretorius, I.S. 2002. Microbial cellulose utilization: fundamentals and biotechnology. *Microbiology and Molecular Biology Reviews*. **66** (3): 506-577.

Mae, K., Hasegawa, I., Sakai, N. and Miura, V. 2000. A new conversion method for recovering valuable chemicals from oil palm shell wastes utilizing liquid-phase oxidation with H_2O_2 under milled conditions. *Energy and fuels*. **14**: 1212-1218.

Maretto, M., Blanchi, F., Vignola, R., Canepari, S., Baric, M., Iazzoni, R., Tagliabue, M. and Papini M.P. 2014. Microporous and mesoporous materials for the treatment of wastewater produced by petrochemical activities. *Journal of Cleaner Production*, **77**: 22-34.

Maroun, R. and Fadel, M.E. 2007. Start-up of anaerobic digestion of source-sorted organic municipal solid waste in the absence of classical inocula. *Environmental Science & Technology*. **41**: 6808–6814.

Martín, B.M.M., Pérez, S.J.A., Fernández, A.F.G., Sánchez, G.J.L., López, C.J.L. and Rodríguez, M.S. 2008. A kinetics study on the biodegradation of synthetic wastewater simulating effluent from an advanced oxidation process using Pseudomonas putida CECT 324. *Journal of Hazardous Materials*. **151**: 780–788.

Mata-Alvarez, J. 2002. *Biomethanization of the Organic Fraction of Municipal Solid Wastes*. Lancaster PA: IWA Publishing.

Mata-Alvarez, J., Mace, S. and Llabres, P. 2000. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. *Bioresource Technology*. **74**: 3-16.

Mater, V., Rosa, E.V.C., Berto, J., Corrˆea, A.X.R., Schwingel, P.R. and Radetski, C. M. 2007. A simple methodology to evaluate influence of H_2O_2 and Fe2+ concentrations on the mineralization and biodegradability of organic compounds in water and soil contaminated with crude petroleum. *Journal of Hazardous Materials*. **149**: 379–386.

Métayer, P. L., Grice, K., Chow, C.N., Caccetta, L., Maslen, E., Dawson, D., Fusetti L. 2014. The effect of origin and genetic processes of low molecular weight aromatic hydrocarbons in petroleum on their stable carbon isotopic compositions. *Organic Geochemistry*. **72**: 23-33.

Metcalf and Eddy Inc. 1991. *Wastewater engineering, treatment, disposal and reuse*. 3rd ed. Singapore: McGraw-Hill, Inc.; p.359–440.

Metcalf and Eddy, 2003. *Wastewater Engineering: Treatment and Reuse*. 4th ed. New York: McGraw Hill, Inc.

Milton, N.G.N. 2008. Homocysteine Inhibits Hydrogen Peroxide Breakdown by Catalase. *The Open Enzyme Inhibition Journal*. **1**: 34-41.

Mitozo, P.A., De-Souza, L.F., Neckel, G.L., Flesch, S., Maris, A.F., Figureueiredo, C.P., Santos, A.R.S.D., Farina, M. and Dafre, A.L. 2011. A study of the relative importance of the peroxiredoxin-, catalase- and glutathione-dependent systems in neural peroxide metabolism. *Free Radical Biology & Medi*. **51**: 69–77.

Moletta, R. and Escoffier, Y. 1994. On line automatic control system for monitoring an anaerobic fluidized bed reactor: response to organic overloads. *Water Science and Technology*. **30**: 11-20.

Moller, H.B., Sommer, S.G. and Ahring, B.K. 2004a. Biological degradation and greenhouse gas emissions during pre-storage of liquid animal manure. *Journal of Environmental Quality*. **33**: 27–36.

Moller, H.B., Sommer, S.G. and Ahring, B.K. 2004b. Methane productivity of manure, straw and solid fractions of manure. *Biomass & Bioenergy*. **26**: 485–495.

Montañés, R., Pérez, M. and Solera, R. 2014. Anaerobic mesophilic co-digestion of sewage sludge and sugar beet pulp lixiviation in batch reactors: Effect of pH control. *Chemical Engineering Journal*. **255**: 492-499.

Mosey, F.E. 1983. Mathematical modeling of the anaerobic digestion process: regulatory mechanisms for the formation of short-chain volatile acids from glucose. *Water Science and Technology*. **21**: 187-196.

Mosey, F.E. and Fernandez, X.A. 1989. Patterns of hydrogen in biogas from the anaerobic digestion of milk-sugars. *Water Science and Technology*. **21**: 187-196.

Mumme, J., Linke, B. and Tolle, R. 2010. Novel upflow anaerobic solid-state (UASS) reactor. *Bioresource Technology*. **101**: 592–599.

Nayono, S.E., Gallert, C. and Winter, J. 2010. Co-digestion of press water and food waste in a biowaste digester for improvement of biogas production. *Bioresource Technology*. **101**: 6987–6993.

Niu, Q., Hojo, T., Qiao, W., Qiang, H. and Li, Y.Y. 2014. Characterization of methanogenesis, acidogenesis and hydrolysis in thermophilic methane fermentation of chicken manure. *Chemical Engineering Journal*. **244**: 587-596.

Niu, Q., Qiao, W., Qiang, H., Hojo, T. and Li Y.Y. 2013. Mesophilic methane fermentation of chicken manure at a wide range of ammonia concentration: Stability, inhibition and recovery. *Bioresource Technology*. **137**: 358-367.

Oh, B.T., Seo, Y.S., Sudhakar, D., Choe, J.H., Lee, S.M., Park, Y.J. and Cho M. 2014. Oxidative degradation of endotoxin by advanced oxidation process (O_3/H_2O_2 & UV/H_2O_2). *Journal of Hazardous Materials*. **279**: 105-110.

OPEC, 2011. The Impact of Recent Events on the Oil Market. Organization of the Petroleum Exporting Countries (OPEC). Vienna: Helferstorferstrasse.

Ortner, M., Rachbauer, L., Somitsch, W., and Fuchs, W. 2014. Can bioavailability of trace nutrients be measured in anaerobic digestion? *Applied Energy*. **126**: 190-198.

Ozturk, I., Irmak, S., Hesenov, A. and Erbatur, O. 2010. Hydrolysis of kenaf (Hibiscus cannabinus L.) stems by catalytical thermal treatment in subcritical water. *Biomass and Bioenergy*. **34** :1578–1585.

Pain, B.F. and Hepherd, R.Q. 1985. Anaerobic digestion of livestock wastes. In: Pain, B.F.,Hepherd, R.Q. (Eds.), Anaerobic Digestion of Farm Waste. *NIRD Technical Bulletins*. Reading, pp. 9–14.

Panorama, Water in fuel production. http//:www.ifpenergiesnouvelles.com, 2011(accessed 1.11.2013).

Parilti, N. B. 2010. Treatment of Petrochemical industry wastewater by a solar oxidation process using the Box-Wilson experimental design method. *Ekoloji*. **19** (77) : 9-15.

Patel, H. and Madamwar, D. 2001. Single and multichamber fixed film anaerobic reactors for biomethanation of acidic petrochemical wastewater-systems performance. *Process Biochemistry*. **36**: 613-619.

Patel, H. and Madamwar, D. 2002. Effects of temperatures and organic loading rates on biomethanation of acidic petrochemical wastewater using anaerobic upflow fixed-film reactor. *Bioresource Technology*. **82**: 65-71.

Prabir, G., Samanta, A.S. and Ray, S. 2011. Kinetics based on mechanism of COD reduction for industrial effluent in Fenton process. *International journal of Chemical Technology*. **3** (1): 26-36.

Rajagopal R., Daniel I. Massé and Singh, G., 2013. A critical review on inhibition of anaerobic digestion process by excess ammonia. *Bioresource Technology*. **143**: 632-641.

Rao, M.S. and Singh, S.P. 2004. Bioenergy conversion studies of organic fraction of MSW: kinetic studies and gas yield–organic loading relationships for process optimization. *Bioresource Technology*. **95**: 173-185.

Ravanchi, M.T., Kaghazchi, T. and Kargari, A. 2009. Application of membrane separation processes in petrochemical industry: a review. *Desalination.* **235**:199–244.

Redzwan, G. and Banks, C.J. 2004. The use of a specific function to estimate maximum methane production in a batch fed anaerobic reactor. *Journal of Chemical Technology and Biotechnology*. **79**: 1174–8.

Redzwan, G. and Banks, C.J. 2007. An evaluation of soft drink wastewater treatment by anaerobic digestion process. *Malaysian Journal of Science.* **26** (1): 35–42.

Ren, T. 2009. *Petrochemicals from Oil, Natural Gas, Coal and Biomass: Energy Use, Economics and Innovation.* PhD Thesis. Utrecht University, Netherland.

Richard, S. 2010. Marine Biogeochemistry: The Invisible Hand Behind. A Vast Carbon Reservoir. *Science Magazine.* **328**: 1476–1477.

Richards, B.K., Uummings, R.J., White, T.E. and Jewell, W., 1991. Methods for kinetic analysis of methane fermentation in high solids biomass digesters. *Biomass Bioenergy* **1** : 65-73.

Rintala, J.A. 1997. Thermophilic anaerobic treatment of industrial process waters and wastewaters. *Microbiol.* **66** (5): 699–704.

Rittmann, B.E. and McCarty, P.L. 2001. *Environmental Biotechnology: Principles and Applications.* New York: McGraw Hill.

Romano, R.T. and Zhang, R.H. 2008. Co-digestion of onion juice and wastewater sludge using an anaerobic mixed biofilm reactor. *Bioresource Technology*. **99**: 631–637.

Rune, P., Moljord, K., Grande, K. and Holmen, A. 2004. *Compositional analysis of naphtha and reformate. Catalytic naphtha reforming*. USA: CRC Press. pp. 2. Retrieved 2010-02-03.

Sahlstrom, L. 2003. A review of survival of pathogenic bacteria in organic waste used in biogas plants. *Bioresource Technology*. **87**: 161–166.

Sankaran, K., Premalatha, M., Vijayasekaran, M. and Somasundaram, V. T. 2014. DEPHY project: Distillery wastewater treatment through anaerobic digestion and phycoremediation—A green industrial approach. *Renewable and Sustainable Energy Reviews*. **37**: 634-643.

Sawayama, S., Tada, C., Tsukahara, K. and Yagishita, T. 2004. Effect of ammonium addition on methanogenic community in a fluidized bed anaerobic digestion. *Journal of Bioscience and Bioengineering*. **97** (1): 65–70.

Schmidt, J. E. and Ahring, B.K. 1993. Effect of Hydrogen and Formate on the degradation of propionate and butyrate in thermophilic granules from an upflow anaerobic sludge blanket. *Applied and Environmental Microbiology*. **59**: 2546-2551.

Serrano A., Siles J. A., Chica A. F. and Martín M. A. 2013. Agri-food waste valorization through anaerobic co-digestion: fish and strawberry residues. *Journal of Cleaner Production*. **54**: 125-132.

Shavisi, Y., Sharifnia, S., Zendehzaban, M., Mirghavami, M.L. and Kakehazar S. 2014. Application of solar light for degradation of ammonia in petrochemical wastewater by a floating TiO2/LECA photocatalyst. *Journal of Industrial and Engineering Chemistry*. **20**: 2806-2813.

Siddique, M.N. I., Munaim, M.S.A. and Zularisam, A.W. 2014. Mesophilic and thermophilic biomethane production by co-digesting pretreated petrochemical wastewater with beef and dairy cattle manure. *Journal of Industrial and Engineering Chemistry*. **20**: 331-337.

Siedlecka, E.M. and Stepnowski, P. 2005. Phenols degradation by Fenton reaction in the presence of chlorides and sulfates. *Polish Journal of Environmental Studies*. **14**: 823 - 828.

Siegrist, H., Vogt, D., Garcia-Heras, J.L. and Gujer, W. 2002. Mathematical model for meso- and thermophilic anaerobic sewage sludge digestion. *Environmental Science & Technology*. **36**: 1113–1123.

Silvestre, G., Illa, J., Fernández, B. and Bonmatí, A., 2014. Thermophilic anaerobic co-digestion of sewage sludge with grease waste: Effect of long chain fatty acids in the methane yield and its dewatering properties. *Applied Energy*. **117**: 87-94.

Smet, E., Van-Langenhove, H. and De-Bo, I. 1999. The emission of volatile compounds during the aerobic and the combined anaerobic/aerobic composting of biowaste. *Atmospheric Environment*. **33**: 1295–1303.

Socías, E.D.T., Calderero, I.F., Oller, I., Lozano, M.J.T., Yuste, F.J. and Malato, S. 2013. Cork boiling wastewater treatment at pilot plant scale: Comparison of solar photo-Fenton and ozone (O_3, O_3/H_2O_2). Toxicity and biodegradability assessment. *Chemical Engineering Journal.* **234**: 232-239.

Solli, L., Bergersen, O., Sørheim, R. and Briseid, T., 2014. Effects of a gradually increased load of fish waste silage in co-digestion with cow manure on methane production. *Waste Management*. **34** :1553-1559.

Song, Y.C., Kwon, S.J. and Woo, J.H. 2004. Mesophilic and thermophilic temperature co-phase anaerobic digestion compared with single-stage mesophilic- and thermophilic digestion of sewage sludge. *Water Research.* **38**: 1653–1662.

Speece, R.E. and Kem, J.A. 1970. The effect of short-term temperature variations on methane production. *Journal of the Water Pollution Control Federation.* **42**: 1990–1997.

Sponza, D.T. and Gok, O. 2010. Effect of rhamnolipid on the aerobic removal of polyaromatic hydrocarbons (PAHs) and COD components from petrochemical wastewater. *Bioresource Technology.* **101**: 914–924.

Srinivasa, R.P.S., Yamada, Y. and Leung, K.Y. 2003. A major catalase (KatB) that is required for resistance to H_2O_2 and phagocyte-mediated killing in Edwardsiella tarda. *Microbiology* (Reading, Engl.) **149** (Pt 9): 2635–2644.

Stafford, D.A. 1982. The effects of mixing and volatile fatty acid concentrations on anaerobic digester performance. *Biomass.* **2**: 43–55.

Strong, G.E. and Cord-Ruwisch, R. 1994. An in situ dissolved hydrogen probe for monitoring anaerobic digesters under overloading conditions. *Biotechnology and Bioengineering.* **45**: 63-68.

Sumathi, S. 2004. *Removal of residue oil from palm oil mill effluent using Chitosan.* Master’s thesis. Universiti Sains Malaysia, Malaysia.

Sun Y., Zhang Y. and Quan X., 2008. Treatment of petroleum refinery wastewater by microwave-assisted catalytic wet air oxidation under low temperature and low pressure. *Separation and Purification Technology*. **62**: 565-570.

Switzenbaum, M.S. and Giraldo-Gomez, E. 1990. Monotoring of the anaerobic methane fermentation process. *Enzyme and Microbial Technology*. **12**: 727-730.

Tafdrup, S. 1995. Viable energy production and waste recycling from anaerobic digestion of manure and other biomass materials. *Biomass & Bioenergy*. **9**: 303–314.

Tatara, M., Makiuchi, T., Ueno, Y., Goto, M. and Sode, K. 2008. Methanogenesis from acetate and propionate by thermophilic down-flow anaerobic packed-bed reactor. *Bioresource Technology*. **99**: 4786–4795.

Tchobanoglous, G., Burton, F.L. and Stensel, H.D. 2003. *Wastewater Engineering: Treatment and Reuse*. 4th ed. New York: McGraw Hill Companies, Inc.

Theofanous, E., Kythreotou, N., Panayiotou, G., Florides, G. and Vyrides I. 2014. Energy production from piggery waste using anaerobic digestion: Current status and potential in Cyprus. *Renewable Energy*. **71**: 263-270.

Tian, Y., Ji, C., Wang K. and Le-Clech P. 2014. Assessment of an anaerobic membrane bio-electrochemical reactor (AnMBER) for wastewater treatment and energy recovery. Journal of Membrane Science 450 : 242-248.

Toerien, D. F. and Hattingh, W.H.J. 1969. Anaerobic digestion I. The microbiology of anaerobic digestion. *Water Research.* **3**: 385-416.

Tommasi T., Ruggeri B. and Sanfilippo S., 2013. Energy valorisation of residues of dark anaerobic production of Hydrogen. *Journal of Cleaner Production*. **34**: 91-97.

Tripathi, S., Pathak, V., Tripathi, D.M. and Tripathi, B.D. 2011. Application of ozone based treatments of secondary effluents. *Bioresource Technology.* **102**: 2481-2486.

UNEP.1994.(Online). http://www.unep.or.jp/ietc/publications/spc/state_of_waste_management/4.asp.(3 April 2012)

UNEP.2002.(Online). http://www.unep.or.jp/ietc/publications/spc/state_of_waste_management/4.asp.(3 April 2012)

UNIDO. 2012. (On line). Renewable Energy in industrial applications. An assessment of 2050.http:www.unido.org/.../Energy.../Energy.../Renewables_%20Industrial (4 April 2012).

Vallejo, M., Román, M.F.S., Ortiz, I. and Irabien, A. 2015. Overview of the PCDD/Fs degradation potential and formation risk in the application of advanced oxidation processes (AOPs) to wastewater treatment. *Chemosphere.* **118**: 44-56.

Van, Lier, J.B., Grolle, K.C.F., Stams, A.J.M., de-Macario, E.C. and Lettinga, G. 1992. Startup of a thermophilic upflow anaerobic sludge bed (UASB) reactor with mesophilic granular sludge. *Applied Microbiology and Biotechnology*. **37**: 130–135.

Vavilin, V.A., Fernandez, B., Palatsi, J. and Flotats, X. 2008. Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview. *Waste Management*. **28**: 939–951.

Veeken, A., Kalyuzhnyi, S., Scharff, H. and Hamelers, B. 2000. Effect of pH and VFA on hydrolysis of organic solid waste. *Journal of Environmental Engineering*. **126** (12): 1076-1081.

Visser, A., Gao, Y. and Lettinga, G. 1993. Effects of short-term temperature increases on the mesophilic anaerobic breakdown of sulfate containing synthetic wastewater. *Water Research*. **27**: 541–550.

Volk, C., Roche, P., Joret, J.C. and Paillard, H. 1996. Comparison of the effect of ozone, ozone-hydrogen peroxide system and catalytic ozone on the biodegradable organic matter of a fulvic acid solution. *Water Research*. **31**: 650-656.

Vrieze, J.D., Hennebel, T., Brande, J.V.D., Bilad, R.M., Bruton, T.A., Vankelecom, I.F.J., Verstraete, W. and Boon, N. 2014. Anaerobic digestion of molasses by means of a vibrating and non-vibrating submerged anaerobic membrane bioreactor. *Biomass and Bioenergy*. **68**: 95-105.

Wang, F. H., Hao, H. T., Sun, R. F., Li, S. Y., Han, R. M., Papelis, C. and Zhang Y. 2014a. Bench-scale and pilot-scale evaluation of coagulation pre-treatment for wastewater reused by reverse osmosis in a petrochemical circulating cooling water system. *Desalination*, **335**: 64-69.

Wang, M., Sun, X., Li, P., Yin, L., Liu, D., Zhang, Y., Li, W. and Zheng, G. 2014b. A novel alternate feeding mode for semi-continuous anaerobic co-digestion of food waste with chicken manure. *Bioresource Technology*. **164**: 309-314.

Wang, Q., Wang, Z., Shen, F., Hu, J., Sun, F., Lin, L., Yang, G., Zhang,Y. and Deng S. 2014c. Pretreating lignocellulosic biomass by the concentrated phosphoric acid plus hydrogen peroxide (PHP) for enzymatic hydrolysis: Evaluating the pretreatment flexibility on feedstocks and particle sizes. *Bioresource Technology*. **166**: 420-428.

Wang, W.M., Song, J. and Han X. 2013. Schwertmannite as a new Fenton-like catalyst in the oxidation of phenol by H_2O_2. *Journal of Hazardous Materials*. **262**: 412-419.

Ward, A.J., Hobbs, P.J., Holliman, P.J. and Jones, D.L. 2008. Optimization of the anaerobic digestion of agricultural resources. *Bioresource Technology*. **99**: 7928-7940.

Wastewater Management technologies in petrochemical industries (On line). http:www.waterworld.com/.../industrial.../wastewater-management-in-the (4 April 2012).

Wu, W. 2007. Anaerobic Co-digestion of Biomass for Methane Production: Recent Research Achievements (On line). htpp:// home.eng.iastate.edu/~tge/ce421-521/wei.pdf. (14April 2012).

Xing, W., Ngo, H.H., Guo, W., Wu, Z., Nguyen, T.T., Cullum, P., Listowski, A. and Yang, N. 2010. Enhancement of the performance of anaerobic fluidized bed bioreactors (AFBBRs) by a new starch based flocculant. *Separation Purification Technology*. **72**: 140–146.

Xing,W., Chen, X., Zuo, J., Wang, C., Lin, J. and Wang, K. 2014. A half-submerged integrated two-phase anaerobic reactor for agricultural solid waste codigestion. *Biochemical Engineering Journal*. **88** :19-25.

Yang, C.F., Wang, C.C. and Tseng, C.H. 2013. Methylamine removal using mixed bacterial strains in a continuous stirred tank reactor (CSTR) system. *International Biodeterioration & Biodegradation.* **85**: 583-586.

Yanto, D.H.Y. and Tachibana, S. 2014. Potential of fungal co-culturing for accelerated biodegradation of petroleum hydrocarbons in soil. *Journal of Hazardous Materials.* **278**: 454-463.

Yeh, C.K.J., Hsu, C.Y., Chiu, C.H. and Huang, K.L. 2008. Reaction efficiencies and rate constants for the goethite-catalyzed Fenton-like reaction of NAPL-form aromatic hydrocarbons and chloroethylenes. *Journal of Hazardous Materials.* **151**: 562–569.

Yeh, C.K.J., Wu, H.M. and Chen, T.C. 2003. Chemical oxidation of chlorinated non-aqueous phase liquids by hydrogen peroxide in natural sand systems. *Journal of Hazardous Materials.* **96**: 29–51.

Yenigün, O. and Demirel, B. 2013. Ammonia inhibition in anaerobic digestion: A review. *Process Biochemistry*. **48**: 901-911.

Yu, B., Xu, J., Yuan, H., Lou, Z., Lin, J. and Zhu N. 2014. Enhancement of anaerobic digestion of waste activated sludge by electrochemical pretreatment. *Fuel*, **130**: 279-285.

Yu, D., Kurola, J.M., Lähde, K., Kymäläinen, M., Sinkkonen, A., and Romantschuk M. 2014. Biogas production and methanogenic archaeal community in mesophilic and thermophilic anaerobic co-digestion processes. *Journal of Environmental Management*, **143** :54-60.

Zhang, A. and Li Y. 2014. Removal of phenolic endocrine disrupting compounds from waste activated sludge using UV, H_2O_2, and UV/H_2O_2 oxidation processes: Effects of reaction conditions and sludge matrix. *Science of The Total Environment*. **493**: 307-323.

Zhang, C., Li J., Liu, C., Liu, X., Wang, J., Li S., Fan, G. and Zhang, L., 2013. Alkaline pretreatment for enhancement of biogas production from banana stem and swine manure by anaerobic codigestion. *Bioresource Technology*. **149**: 353-358.

Zhang, H., Choi, H.J. and Huang, C.P. 2006. Treatment of landfill leachate by Fenton's reagent in a continuous stirrer tank reactor. *Journal of Hazardous Materials*. **136**: 618-623.

Zhang, W., Wei, Q., Wu, S., Qi, D., Wei Li, W., Zuo, Z. and Dong R. 2014a. Batch anaerobic co-digestion of pig manure with dewatered sewage sludge under mesophilic conditions. *Applied Energy*. **128**: 175-183.

Zhang, W., Yong,Y., Zhang, G., Yang, C. and Mao, Z. 2014b. Mixing Characteristics and Bubble Behavior in an Airlift Internal Loop Reactor with Low Aspect Ratio. *Chinese Journal of Chemical Engineering*. **22**: 611-621.

Zhang, C., Haijia Su, H., Baeyens, J. and Tan T. 2014c. Reviewing the anaerobic digestion of food waste for biogas production. *Renewable and Sustainable Energy Reviews*, **38**: 383-392.

Zhou, T, Li, Y., Wong, F.S. and Lu, X. 2008. Enhanced degradation of 2,4-dichlorophenol by ultrasound in a new Fenton like system (Fe/EDTA) at ambient circumstance. *Ultrason. Sonochem.* **15**: 782–790.

Zhou, T., Li, Y., Ji, J., Wong, F.S. and Lu, X. 2008. Oxidation of 4-chlorophenol in a heterogeneous zero valent iron/H_2O_2 Fenton-like system: kinetic, pathway and effect factors. *Separation and Purification Technology*. **62**: 553–560.

Zhu J., Zheng, Y., Xu, F. and Li, Y., 2014. Solid-state anaerobic co-digestion of hay and soybean processing waste for biogas production. *Bioresource Technology* **154**: 240-247.

Zhuang, H., Han, H., Jia, S., Hou, B. and Zhao, Q. 2014. Advanced treatment of biologically pretreated coal gasification wastewater by a novel integration of heterogeneous catalytic ozonation and biological process. *Bioresource Technology*, **166**: 592-595.

Zuikes, J.G. 1980. *Microbial population in digester*. London: Applied Sciences publisher limited.

APPENDIX A

Cost Estimation for treating 1 L PWW by chemo-biological method

Power = Voltage X Current

Current = Energy usage X Power factor

- Energy usage (Pumps) = 2.2 A
- Voltage usage = 120 V
- Power factor = 0.8
- Operation = 24 h
- Working volume = 2.7 L / d
- Energy value = @ USD 0.3 / KW-h

Estimated Power consumption = Voltage X Current
= [(120 X 2.2 X 0.8) / 1000] X 24
= 5.07 KW-h

Estimated cost / d = 5.07 KW-h X USD 0.3 / KW-h
= USD 1.52 / d

Estimated cost / L treated = (USD 1.52 / d) / (2.7 L / d)
= USD 0.56 / L

Estimated chemical demand / L/ d = USD 0.07 / L

Total Estimated Cost = Power cost + Chemical cost
= USD 0.56 + USD 0.07
= USD 0.63 / L / d

APPENDIX B

Basic Calculations of Reactor Performance Parameters

Sample Calculation

$$Q = \frac{V_W}{HRT}$$

- Flow rate, $Q = 0.27$ L /d
- Working Volume, $V_W = 2.7$ L

$$HRT = V_W / Q$$

$$= 2.7 \text{ L} / 0.27 \text{ L /d}$$

$$= 10 \text{ d}$$

$$HRT = \frac{COD_{in}}{OLR}$$

- HRT = 10 d
- Influent COD, $COD_{in} = 63.1$ g / L

$$OLR = (63.1 \text{ g / L}) / 10 \text{ d}$$

$$= 6.31 \text{ g / L}$$

$$\frac{F}{M} = \frac{COD_{in}}{VSS * HRT}$$

- Volatile suspended solid, VSS = 25.24 g COD / L / d
- Organic loading rate, OLR = 6.31 g / L

$$F/M = OLR / VSS$$

$$= (6.31 \text{ g / L}) / 25.24 \text{ g COD / L / d}$$

$$= 0.25$$

APPENDIX C

EFFECT OF OLR ON COD REMOVAL FROM PWW

Days	**OLR (Kg COD/m^{3}d)**	**n**	**COD removal (%)**	
			Mean	**±SD**
5	3	3	40	2
10	4	3	45	3
15	5	3	48	1.5
20	6	3	50	2
25	7	3	52	1.3
30	8	3	55	3
35	9	3	57	2
40	10	3	65	1
45	11	3	70	1.5
50	12	3	75	2
55	13	3	80	1.7
60	14	3	85	2.5
65	15	3	90	3
70	16	3	95	2
75	17	3	96	1
80	18	3	97	2
85	19	3	98	1.5
90	20	3	93	2
95	21	3	96	3
100	22	3	95	1.7
110	23	3	93	2
115	24	3	93	3
120	25	3	93	1.5
125	26	3	92	2
130	27	3	92	3
135	28	3	92	2
140	28	3	92	1

APPENDIX D

Significant error calculation for finding optimum mixing ratio of PWW and AM for enhanced methane production

Mixing ratio	CH_4 (m^3)	SD	SE
0	4.93	0.4	0.133333
10	5.47	0.6	0.2
20	6.76	0.6	0.2
30	7.3	0.7	0.233333
40	8.5	0.7	0.233333
50	11.07	0.7	0.233333
60	8.85	0.6	0.2
70	7.16	0.6	0.2
80	5.68	0.5	0.166667
90	3.73	0.4	0.133333
100	3.1	0.4	0.133333

APPENDIX E

Significant error calculation for finding optimum flow rate of PWW and AM for enhanced methane production

Flow rate (mL/d)	CH_4 (m^3)	SD	SE
150	0.62	0.05	0.016667
170	0.7	0.05	0.016667
220	0.65	0.05	0.016667
300	0.6	0.05	0.016667
370	0.55	0.04	0.013333
410	0.51	0.04	0.013333
475	0.46	0.04	0.013333
540	0.42	0.04	0.013333
600	0.35	0.03	0.01
640	0.31	0.03	0.01
680	0.29	0.03	0.01

APPENDIX F

Significant error calculation for finding optimum f/m ratio of PWW and AM for enhanced methane production

F/M	CH_4 (mL/g VS)	SD	SE
0.25	451.9	15	5
0.5	461.5	17	5.66667
0.75	419.8	10	3.33333
1	390.6	8	2.66667
1.25	374.6	8	2.66667
1.5	353.13	7	2.33333
1.75	327.6	7	2.33333
2	301.92	6	2

APPENDIX G

Figure 3.4 Photograph of Batch test

APPENDIX H

Figure 3.5 Photograph of activated sludge biomass

APPENDIX I

Physicochemical individualities of decomposed slurry

	Solid portion		**Water portion**
	50%PWW+50%AM		**50%PWW+50%AM**
Recovery of sludge (m^3 sludge m^{-3} substrate)	0.08	Recovery of water (m^3water m^3substrate)	0.85
Moisture (%)	95.95	COD (g/L)	0.12
		Turbidity (unfiltered turbidity, UNFT)	1290
		Suspended solids (g/L)	0.05

APPENDIX J

Fiscal viability of ACD and AD factory

	50% PWW/50% AM	**PWW**
Waste for treatment (m^3y^{-1})	11556	18995
Electrical engine (kWe)	85.4	329.2
Electricity generation (MWh/year)	340.42	581.4
Thermal energy potential (GJ/year)	1221.12	2091.9
Required Heat for AD plant (GJ/year)	1968.80	3187.75
Water heating capacity (m^3/year)	3390.53	3395.54
System cost ($)	493788	1341752.79
Yearly Income ($)	223643	296901
Yearly cost ($)	91966	206790
Yearly benefits ($)	131677	90111
Payback period (yrs)	3.75	14.89
GCW (Million of Dollars)	0.78	-
IRR (%)	27	-

APPENDIX K

Methane and Biogas production during 109 days

CH_4 (m^3/m^3d)	Biogas (m^3/m^3d)
4.22	5.7
4.86	6
4.9	5.8
4.88	6
4.9	5.8
4.92	6
4.9	5.8
4.92	6
4.9	5.8
5	6
5.4	6.7
5.64	6.8
5.5	7
5.64	6.8
5.5	7
5.64	6.8
5.5	7
5.64	6.7
5.6	7
5.65	8.7
6.6	9
6.7	8.8
6.75	9
6.7	8.8
6.75	9
6.7	8.8
6.75	9
6.7	8.8
6.75	9
6.7	9.8
7	10
7.2	9.8
7	10
7.1	9.8
7.2	10
7	9.8
7.2	10

7	9.8
7.2	10
7	11.8
8.2	12
8.4	11.8
8.2	12
8.4	11.8
8.2	12
8.4	11.8
8.2	12
8.4	11.8
8.2	12
8.4	13.8
10.8	14
11.06	13.8
10.8	14
11.06	13.8
10.8	14
11.06	13.8
10.8	14
11.06	13.8
10.8	14
11.06	13.8
9.9	12.8
10.2	13
10	12.8
10.2	13
10	12.8
10.2	13
10	12.8
10.2	13
10	12.8
10.2	13
7	10.7
7.15	11
7	10.8
7.15	11
7	10.8
7.15	11
7	10.8
7.15	11
7	10.8

7.15	11
5.5	10.8
5.67	11
5.5	8.7
5.67	9
5.5	8.8
5.67	9
5.5	8.8
5.67	9
5.5	8.8
5.67	9
5.5	8.8
3.6	9
3.72	5.8
3.6	6
3.72	5.8
3.6	6
3.72	5.8
3.6	6
3.72	5.8
3.6	6
3.72	5.8
2.9	6
3	5.8
2.9	6
3	4.8
2.9	5
3	4.8
2.9	5
2.5	4.8
2.4	5
2.3	4.8

BIODATA OF THE AUTHOR

The author was born in 1984 in Jessore, Bangladesh. He obtained bachelor degree in Civil Engineering in 2007 from Khulna University of Engineering & Technology, Bangladesh. Subsequently, Mr. Nurul obtained Master degree in Civil Engineering in 2012 from University Malaysia Pahang, Malaysia. Currently he is perusing his Ph.D. Program at the Faculty of Civil Engineering & Earth Resources, University Malaysia Pahang.

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LIST OF PUBLICATIONS

1. **Md. Nurul Islam Siddique,** Mimi Sakinah Abdul Munaim, Zularisam, A.W. 2014. Mesophilic and thermophilic biomethane production by co-digesting pretreated petrochemical wastewater with beef and dairy cattle manure. *Journal of Industrial and Engineering Chemistry 20 (2014) 331–337. Elsevier.* **Impact Factor-2.15.**

2. **Md. Nurul Islam Siddique,** Mimi Sakinah Abdul Munaim, Zularisam, A.W. 2014. Feasibility analysis of anaerobic co-digestion of activated manure and petrochemical wastewater in Kuantan (Malaysia). *Journal of Cleaner Production, In Press, Corrected Proof, Available online 19 August 2014. Elsevier.* **Impact Factor-3.6.**

3. **Md. Nurul Islam Siddique,** Mimi Sakinah Abdul Munaim, Zularisam, A.W. 2014. Influence of flow rate variation on bio-energy generation during anaerobic co-digestion. *Journal of Industrial and Engineering Chemistry. Elsevier (Accepted on 18 Dec, 2014).* **Impact Factor-2.15.**

4. **Md. Nurul Islam Siddique**, Mimi Sakinah Abdul Munaim, Zularisam, A.W. 2014. Influence of food to microbe ratio variation on bio-energy production during anaerobic co-digestion. *Applied Energy (Under review). Elsevier.* **Impact Factor-5.26.**

5. **Md. Nurul Islam Siddique**, Mimi Sakinah Abdul Munaim, Zularisam, A.W. 2014. Role of biogas recirculation in enhancing Petrochemical wastewater treatment efficiency of continuous stirred tank reactor. *Journal of Cleaner Production, In Press, Accepted Manuscript, Available online 18 December 2014, Elsevier.* **Impact Factor-3.6.**

6. **Md. Nurul Islam Siddique**, Mimi Sakinah Abdul Munaim, Zularisam, A.W. 2014. Role of hydraulic retention time in enhancing bio-energy generation from Petrochemical wastewater. *Journal of Cleaner Production, (Under review) Elsevier.* **Impact Factor-3.6.**

7. **Md. Nurul Islam Siddique,** M. Sakinah, A.W. Zularisam, 2014. Enhanced production of renewable methane from petrochemical wastewater using CSTR. International conference on advances in applied science and environmental engineering, ASEE-2014, *02-03August, 2014,* Kualalampur, Malaysia.

8. **Md. Nurul Islam Siddique,** M. Sakinah, A.W. Zularisam, 2014. Anaerobic bioconversion of methane from petrochemical wastewater using CSTR. International conference on energy and environmental sciences, ICEES-2014, 04-05, Sep, 2014, Kualalampur, Malaysia.

9. **Md. Nurul Islam Siddique,** M. Sakinah, A.W. Zularisam, 2014. Intensified CSTR for bio-methane generation from petrochemical wastewater. International conference on chemical, environmental and biological sciences, CEBS-2014, 17-18, Sep, 2014, Kualalampur, Malaysia.

10. **Md. Nurul Islam Siddique,** M. Sakinah, A.W. Zularisam, 2014. Biomethane generation potential of petrochemical wastewater by anaerobic digestion using CSTR. 4th Johor Biotechnology Conference and Exhibition, 2014, 26-27 August, 2014, Johor, Malaysia.