STUDY ON THE EFFECTS OF CHELATING AGENTS ON HYDRAULIC RETENTION TIME, METHANE AND HYDROGEN SULPHIDE GAS YIELDS FROM SOLID WASTES.

BY

NWOKEM NSIDIBEABASI CALVIN
B.Sc. (ABU), 2004; M.Sc. (ABU), 2008.
Ph.D/SCIE/9825/2009-2010.

A DISSERTATION WRITTEN IN THE DEPARTMENT OF CHEMISTRY AND SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE IN ANALYTICAL CHEMISTRY OF

AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA.

OCTOBER, 2014

DECLARATION

I hereby declare that the work in this dissertation titled **"Study on the Effects of Chelating Agents on Hydraulic Retention Time, Methane and Hydrogen Sulphide Gas Yields from Solid Wastes"** was performed by me in the Department of Chemistry under the supervision of Prof. C.E. Gimba, Prof. G.I. Ndukwe and Dr E.E. Ella. The information derived for the literature has been duly acknowledged in the text and list of references provided. No part of this work has been presented for another degree at any institution. I take exclusive responsibility for its strength and weaknesses.

....

NWOKEM NSIDIBEABASI CALVIN **DATE**

CERTIFICATION

This dissertation tilted "**Study on the Effects of Chelating Agents on Hydraulic Retention Time, Methane and Hydrogen Sulphide Gas Yields from Solid Wastes"** meets the requirements governing the award of degree of Doctor of Philosophy (PhD) of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

....
Prof. C.E. Gimba, Date
Chairman, Supervisory Committee,
Department of Chemistry,
ABU,
Zaria.

....
Prof. G.I. Ndukwe, Date
Member, Department of Chemistry,
Ahmadu Bello University,
Zaria.

....
Dr. E.E. Ella, Date
Member, Department of Microbiology,
Ahmadu Bello University,
Zaria.

....
Prof. V.O. Ajibola, Date
Head,
Department of Chemistry,
Ahmadu Bello University,
Zaria.

....
Prof. A. Z. Hassan, Date
Dean,
School of Postgraduate Studies,
Ahmadu Bello University,
Zaria.

DEDICATION

This work is dedicated to my Lord Jesus, who has been my Teacher, my Guide and my King; and to my late father, Dr E.S. Essien.

ACKNOWLEDGEMENT

I wish to acknowledge with deep gratitude the grace of God that has made it possible for me to complete this work. The Almighty Who is on the throne is in control and He has made this work a reality. Thank you Lord.

I also like to express my sincere gratitude to my supervisors, Prof. C.E. Gimba, Prof. G.I. Ndukwe and Dr. E.E. Ella, for the valuable guidance and the continuous help they generously provided throughout the course of this work. I wish to appreciate the Lord for the life of late Prof. J.F. Iyun and late Prof. J.A. Kagbu who never ceased to challenge me with regards to my academic pursuits. May the good Lord continue to fill the vacuum their sudden departure created in the lives of their family members and colleagues.

My appreciation goes to the Vice Chancellor, Ahmadu Bello University, Zaria, Prof. Abdullahi Mustapha, for approving the purchase of the Biogas analyser used for this research work.

Many thanks to the Management of The National Animal Production Research Institute Zaria (NAPRI), for making available some of the materials needed for this research work.

A special thank goes to Prof. A.J. Nok, for assisting with the purchase of the reagents used for this research work and also to Dr. B.D. Lawong of the Department of Economics, Ahmadu Bello University, Zaria, for his assistance with the Statistical analysis of this work.

I also wish to specially acknowledge the Head, Department of Chemistry, Prof. V.O. Ajibola for his assistance and moral support in making the completion of this programme a reality. To the staff members of the Department of Chemistry, I say a big

thank you; especially the Chief Technologist Mrs O.R.A. Iyun and other technical staff in the department who have at one time or the other helped me out of difficult situations. To Mr Yakubu Apeh of the Department of Biochemistry, I say a big thank you for your kind assistance in the use of facilities under your care. My appreciation also goes to Mrs Bukky Patrick for always assisting with my laboratory work. Thanks to Pst. S.A. Ekwuribe and Alhaji Bashiru, for the use of facilities at the Multi-User Science Research Laboratories, Ahmadu Bello University, Zaria. Special thanks go to Mr G.A. Shallangwa and Mrs S. A. Hamza for helping with my chemistry drawings and also to Dr Mathias Chia for assisting with the International transactions.

I would also like to specially thank my mother, Mrs B.E. Essien; Mama, if not for your help l would not have as much as began my laboratory work. May God continue to bless you with sound health and long life. To my brothers and Sisters, Mr Emmanuel Essien, Prof. and Dr. (Mrs) Dikki, Sister Joy, Mary, Mrs Emem Ezenwa, Hannah, Ruth, Grace and Gift I am grateful to you all for the support and assistance. May the good Lord continue to bless and take you all higher.

My special thanks go to my husband Dr. Calvin Onyedika Nwokem. Sweetheart, you are really a blessing indeed, thank you for helping me come up with this topic, and painstakingly typing my work alongside helping with the household chores; you are really God's perfect will for my life. God bless. To our kids Sylvia (Ima) and Chrystabelle (Imabong), you both are simply wonderful, sorry you had to make do with my absence at some point or the other during the course of this work. May the Lord bless you both.

To all my friends and well wishers too numerous to mention, may God bless.

TABLE OF CONTENTS

Title Page		i
Declaration		ii
Certification		iii
Dedication		iv
Acknowledgement		v
Table of Contents		vii
List of Tables		xii
List of Figures		xiii
List of Plates		xvii
List of Appendices		xviii
Abbreviations		xix
Abstract		xxi

CHAPTER ONE

1.0	**INTRODUCTION**	1
1.1	Statement of Problem	1
1.2	Fuels and their Characteristics	3
1.2.1	Fossil Fuels	3
1.2.2	Solar Fuels	4
1.2.3	Biofuels	5
1.3	Anaerobic Digestion	6
1.4	Substrates for Anaerobic Digestion	7
1.5	Justification for the Study	9
1.6	Aims and Objectives	9

CHAPTER TWO

2.0	**LITERATURE REVIEW**	11
2.1	History and Importance of Biogas	11
2.1.1	Direct Combustion	12
2.1.2	Combined Heat and Power (CHP) Generation	12
2.1.3	Fuel Cells Production	13
2.1.4	Biogas as an Automobile Fuel	14
2.2	Types of Anaerobic Digestion Systems	15
2.2.1	The Batch Systems	15
2.2.2	The Continuously Stirred Systems	16
2.2.3	Two-Stage Systems	16
2.3	The Process involved in Anaerobic Digestion	17
2.4	Factors Affecting Anaerobic Digestion	20
2.4.1	Temperature	20
2.4.2	pH values	22
2.4.3	Available Nutrients	23
2.4.4	Presence of Inhibitors	23
2.4.5	Volatile Fatty Acids (VFA)	24
2.4.6	Hydraulic Retention Time (HRT)	24
2.5	Advantages of the Biogas Technology	25
2.5.1	Mitigation of Green house Effect	25
2.5.2	Reduced Dependence on Imported Fuels	25
2.5.3	Waste Reduction	26
2.5.4	Improved Income for Farmers	26
2.5.5	Fertilizer	26
2.5.6	Job Creation	26
2.6	Principle of Analytical Techniques Employed in Biogas Generation	27

2.6.1 Principle of Elemental Determination using Atomic Absorption Spectrometer (AAS) 27

2.6.2 Principle of Biogas Characterization using Biogas 5000 27

2.7 Techniques Employed for the Enhancement of Biogas 28

2.7.1 Recycling of Digested Slurry 28

2.7.2 Variation of Operational Parameters 29

2.7.3 Use of Biological and/or Chemical Additives 32

CHAPTER THREE

3.0 **MATERIALS AND METHODS** 36

3.1 MATERIALS 36

3.1.1 Sample Collection 36

3.1.2 Sample Preparation 36

3.1.3 Apparatus/ Equipment 36

3.1.4 Reagents and their Preparations 37

3.2 Methods 42

3.2.1 Ash Content Determination 42

3.2.2 Moisture Content Determination 42

3.2.3 Determination of Lipid Content 43

3.2.4 Nitrogen Content Determination (Kjedahl Method) 43

3.2.5 Carbohydrate Content Determination 45

3.2.6 Total Solids Determination 45

3.2.7 Volatile Solid Determination 46

3.2.8 Determination of Trace Metals in Waste Samples 46

3.2.9 Method for Monitoring System Performance 47

3.2.10 Method for production and Measurement of Biogas 47

3.2.11 VFA and pH determination 51

CHAPTER FOUR

4.0	**RESULTS**	52
4.1	Results of the VFA, pH and CH_4 gas Yield Analysis at Optimized Substrate Weights	52
4.2	Proximate Analysis and Trace Metal Determination of Substrates	52
4.3	HRT of Optimized Substrates	58
4.4	Optimization of Chelating Ligand Ability, VFA Concentration and pH values	58
4.5	Effect of Chelating Ligand on HRT	73
4.6	Effect of Increasing Concentration of Chelating Ligand on CH_4 gas yield	73
4.7	Effect of Addition of Chelating Ligand on H_2S Concentration	73
4.8	Effect of Increasing Chelating Ligand Concentration on VFA	101
4.9	Effect of Increasing Concentration of Chelating Ligand on HRT	101
4.10	Co-digestion	101
4.11	Effect of Chelating Ligand on CH_4 gas yield and VFA Concentration in CH: CM Co-digestion	121
4.12	Effect of Increasing Concentration of Chelating Ligand on CH_4 gas Yield and VFA Concentration in Co-digestion Systems	121
4.13	Effect of Addition of Chelating Ligand on HRT in Co-digestion Systems	130
4.14	Effect of Addition of Chelating Ligand on H_2S Concentration Produced within Co-digestion Systems	130

CHAPTER FIVE

5.0	**DISCUSSION**	140
5.1	CH_4 gas yield at Optimized Substrate Weight	140
5.2	VFA Concentration of Optimized Substrates	140
5.3	Effects of Substrate Nutrients on CH_4 gas yield	142
5.4	HRT of Optimized Substrates	144

5.5	Optimization of Chelating Ligand Ability	145
5.6	Effect of Addition of Chelating Ligand on HRT	148
5.7	Effect of Increasing Chelating Ligand Concentration on CH_4 gas yield	152
5.8	Effect of Addition of Chelating Ligand on H_2S Concentration	153
5.9	Effect of Increasing Chelating Ligand Concentration on VFA Concentration	154
5.10	Effect of Increasing Chelating Ligand Concentration on HRT	156
5.11	Co-digestion	157
5.12	Effect of Chelating Ligand on CH_4 gas yield and VFA Concentration in CH: CM Co-digestion Systems	157
5.13	Effect of Increasing Concentration of Chelating Ligand on CH_4 gas Yield and VFA Concentration in Co-digestion Systems	158
5.14	Effect of Addition of Chelating Ligand on HRT in Co-digestion Systems	159
5.15	Effect of Addition of Chelating Ligand on H_2S Concentration Produced within Co-digestion Systems	160

CHAPTER SIX

6.0	**SUMMARY, CONCLUSION AND RECOMMENDATION**	161
6.1	Conclusion	163
6.2	Recommendation	164
REFERENCES		165

LIST OF TABLES

TABLE	TITLE	PAGE
1.1	Types of Waste Materials Suitable for Biogas	8
2.1	Temperature and its Effects on Typical Retention Times	21
2.2	Logarithm Stability Constant of Some Chelating Ligands	35
3.1	Industrial Sites from where each Sample was sourced	36
3.2	Structures and Names of the Chelating Ligands	38
4.1	pH Values of the Substrates GU, BL, CM, CD and CH	55
4.2	Physiochemical Properties of Substrates	53
4.3	Effect of Addition of Chelating Ligand on pH for CM Substrate	67
4.4	Effect of Addition of Chelating Ligand on pH for CM Substrate	68
4.5	Effect of Addition of Chelating Ligand on pH for CM Substrate	69

LIST OF FIGURES

FIGURE	TITLE	PAGE
2.1	Schematic Diagram of a Simple Biogas Fuel Cell	14
2.2	Degradation of Pyruvate (End Product of Glucose Degradation) to Propionic Acid	19
4.1	CH_4 gas Yield at Optimization of Substrate Weight	53
4.2	VFA Concentration of Digester Systems GU, BL, CM, CH and CD at Optimized Substrate Weights	54
4.3	Concentration of Trace Metals within the Various Substrates	57
4.4	HRT for the GU Substrate	59
4.5	HRT for the BL Substrate	60
4.6	HRT for the CD Substrate	61
4.7	HRT for the CM Substrate	62
4.8	HRT for the CH Substrate	63
4.9	Optimization of the Chelating Ligand Ability within the CM Digester	64
4.10	Optimization of the Chelating Ligand Ability within the CD Digester	65
4.11	Optimization of the Chelating Ligand Ability within the CH Digester	66
4.12	Effect of Addition of Chelating Ligand on VFA concentration in CM Digester	67
4.13	Effect of Addition of Chelating Ligand on VFA concentration in CD Digester	68
4.14	Effect of Addition of Chelating Ligand on VFA concentration in CH Digester	69
4.15a	Effect of Addition of Chelating Ligand on HRT in the CM-EDDA and MESYL Digester	74
4.15b	Effect of Addition of Chelating Ligand on HRT in the CM-NTA and PENI Digester	75
4.15c	Effect of Addition of Chelating Ligand on HRT in the CM-EDTA, DTPA and PORPH Digester	76
4.16a	Effect of Addition of Chelating Ligand on HRT in the CD-EDDA and MESYL Digester	77

4.16b Effect of Addition of Chelating Ligand on HRT in the CD-NTA and DTPA Digester 78

4.16c Effect of Addition of Chelating Ligand on HRT in the CD-EDTA, PORPH and PENI Digester 79

4.17a Effect of Addition of Chelating Ligand on HRT in the CH-EDDA and MESYL Digester 80

4.17b Effect of Addition of Chelating Ligand on HRT in the CH-DTPA and PENI Digester 81

4.17c Effect of Addition of Chelating Ligand on HRT in the CH-EDTA, NTA and PORPH Digester 82

4.18a Percentage of CH_4 gas yield from EDDA CM Digester System 83

4.18b Percentage of CH_4 gas yield from NTA CM Digester System 84

4.18c Percentage of CH_4 gas yield from DTPA CM Digester System 85

4.19a Percentage of CH_4 gas yield from EDTA CD Digester System 86

4.19b Percentage of CH_4 gas yield from PORPH CD Digester System 87

4.19c Percentage of CH_4 gas yield from DTPA CD Digester System 88

4.20a Percentage of CH_4 gas yield from EDDA CD Digester System 89

4.20b Percentage of CH_4 gas yield from MESYL CD Digester System 90

4.20c Percentage of CH_4 gas yield from DTPA CD Digester System 91

4.21a H_2S gas Concentration from EDDA CM Digester System 92

4.21b H_2S gas Concentration from NTA CM Digester System 93

4.21c H_2S gas Concentration from DTPA CM Digester System 94

4.22a H_2S gas Concentration from EDTA CD Digester System 95

4.22b H_2S gas Concentration from DTPA CM Digester System 96

4.22c H_2S gas Concentration from PORPH CM Digester System 97

4.23a H_2S gas Concentration from EDDA CH Digester System 98

4.23b H_2S gas Concentration from DTPA CH Digester System 99

4.23c H_2S gas Concentration from MESYL CH Digester System 100

4.24a Concentration of VFA in EDDA CM Digester System 102

4.24b	Concentration of VFA in NTA CM Digester System	103
4.24c	Concentration of VFA in DTPA CM Digester System	104
4.25a	Concentration of VFA in EDTA CD Digester System	105
4.25b	Concentration of VFA in DTPA CD Digester System	106
4.25c	Concentration of VFA in PORPH CD Digester System	107
4.26a	Concentration of VFA in EDDA CH Digester System	108
4.26b	Concentration of VFA in DTPA CH Digester System	109
4.26c	Concentration of VFA in MESYL CH Digester System	110
4.27	Effect of Increasing Chelating Ligand Concentration on HRT in CM EDDA Digester	111
4.28	Effect of Increasing Chelating Ligand Concentration on HRT in CM NTA Digester	112
4.29	Effect of Increasing Chelating Ligand Concentration on HRT in CM DTPA Digester	113
4.30	Effect of Increasing Chelating Ligand Concentration on HRT in CD EDTA Digester	114
4.31	Effect of Increasing Chelating Ligand Concentration on HRT in CD DTPA Digester	115
4.32	Effect of Increasing Chelating Ligand Concentration on HRT in CD PORPH Digester	116
4.33	Effect of Increasing Chelating Ligand Concentration on HRT in CH EDDA Digester	117
4.34	Effect of Increasing Chelating Ligand Concentration on HRT in CH DTPA Digester	118
4.35	Effect of Increasing Chelating Ligand Concentration on HRT in CH MESYL Digester	119
4.36	Percentage of CH_4 gas Produced from the Different Blends	120
4.37	Effect of Addition of Chelating Ligand on Co-digestion	122
4.38	Effect of Chelating Ligands on VFA Concentration in Co-digestion	123

4.39a	Effect of increasing EDDA CH: CM Concentration on CH_4 gas Yield in Co-digestion	124
4.39b	Effect of increasing DTPA CH: CM Concentration on CH_4 gas Yield in Co-digestion	125
4.39c	Effect of increasing MESYL CH: CM Concentration on CH_4 gas Yield in Co-digestion	126
4.40a	Effect of increasing EDDA CH: CM Concentration on VFA Concentration in Co-digestion	127
4.40b	Effect of increasing DTPA CH: CM Concentration on VFA Concentration in Co-digestion	128
4.40c	Effect of increasing MESYL CH: CM Concentration on VFA Concentration in Co-digestion	129
4.41a	Effect of Addition of Chelating Ligand on HRT for Co-digestion in EDDA and NTA	131
4.41b	Effect of Addition of Chelating Ligand on HRT for Co-digestion in EDTA and DTPA	132
4.41c	Effect of Addition of Chelating Ligand on HRT for Co-digestion in MESYL, PORPH and PENI	133
4.42	Effect of Increasing Concentration of Chelating Ligand on HRT in Co-digestion System containing EDDA	134
4.43	Effect of Increasing Concentration of Chelating Ligand on HRT in Co-digestion System containing DTPA	135
4.44	Effect of Increasing Concentration of Chelating Ligand on HRT in Co-digestion System containing MESYL	136
4.45a	Effect of increasing EDDA CH: CM Concentration on H_2S Concentration	137
4.45b	Effect of increasing DTPA CH: CM Concentration on H_2S Concentration	138
4.45c	Effect of increasing MESYL CH: CM Concentration on H_2S Concentration	139
5.1	Equation of Reaction Showing Catalytic Effect of Metal ions in the Formation of Reactive Intermediates during the course of the Anaerobic Digestion Process	151

LIST OF PLATES

PLATES	TITLE	PAGE
3.1.1	Diagram Showing Experimental Setup for the Biogas Production	47
3.1.2	Diagram Showing the Biogas5000 Analyser	48

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
I	Results Showing the Percentage of CH_4 gas yield from the various Weights used	177
II	Results Showing the Effect of Addition of Chelating Liquid on CH_4 gas yield	178
III	Result Showing the Effect of Increasing Chelating Ligand Concentration on CH_4 gas yield	180
IV	Result Showing the Effect of Addition of Chelating Ligand on VFA Concentration	185
V	Result Showing the Effect of Increasing Chelating Ligand Concentration on VFA	187
VI	Result Showing H_2S Concentration in the various Substrates	189
VII	Results Showing the Effect of Increasing Chelating Ligand Concentration on Percentage of H_2S	190
VIII	Results Showing the Percentage of CH_4 Produced from the different Blends in Co-digestion	194
IX	Results Showing the Effect of Addition of Chelating Ligand on CH: CM Co-digestion	195
X	Results Showing the Effect of Increasing Chelating Ligand Concentration on Co-digestion	196
XI	Result Showing the Effect of Addition of Chelating Ligand on VFA Concentration in Co-digestion	198
XII	Result Showing the Effect of Increasing Chelating Ligand Concentration on VFA	199
XIII	Result Showing Effect of Chelating Ligand on H_2S Concentrations in Co-digestion	201

ABBREVIATIONS

HRT	Hydraulic Retention Time
NTA	Nitrilotriacetic acid
DTPA	Diethylenetriamine-pentacetic acid
MESYL	Deferoxamine mesylate
PORPH	5, 10, 15, 20-tetra (4-pyridyl)- 21H, 23H-porphine
PENI	D-penicillamine
EDTA	Ethylene diamine-tetraacetic acid
EDDA	Ethylene diamine-N, N-diacetic acid
VFA	Volatile Fatty Acid
TS	Total solid
VS	Volatile solid
CM	Cow manure
CH	Chicken dung
CD	Cow cud
GU	Guinea corn
BL	Barley
CH:CM	Chicken dung: Cow manure
CH:GU	Chicken dung: Guinea corn
GU:BL	Guinea corn: Barley
CH:BL	Chicken dung: Barley
CM:BL	Cow manure: Barley
CM:GU	Cow manure: Guinea corn
mg/L	Milligram per litre
μM	Micro molar

ppm	Parts per million
AAS	Atomic Absorption Spectrophotometer
G	Gramme

ABSTRACT

This research work focused on addition of chelating ligands in order to enhance the overall digestion process; thereby improving methane gas yield, reducing HRT and decreasing concentration of H_2S produced within digester systems. In view of this, several experiments and analyses such as metal determination, VFA, and CH_4 gas yield were carried out which involved the use of the Atomic Absorption Spectrophotometer (AAS), Biogas 5000 Analyser, and other analytical tools. The results from these analyses showed that, at mesophilic temperature range of 29 – 31^oC, four of the different substrates, GU, BL, CH and CD, recorded maximum CH_4 gas yield of 8.30 %, 33.30 %, 84.80 % and 67.60 % respectively at an optimum weight of 150 g. The only exception observed was with CM substrate which recorded a CH_4 gas yield of 75.40 % at an optimum weight of 250 g. On addition of chelating ligands, EDDA, NTA, EDTA, DTPA, MESYL, PORPH and PENI to the best three performing substrate digesters namely, CM, CD and CH, it was observed that depending on the chelating ligand added and the substrate, an increase in the CH_4 yield was recorded which ranged from 1-15 %. This improvement in CH_4 gas yield was due to the increased bioavailability of essential nutrients on addition of chelating ligands which invariably promoted the growth and stability of the CH_4 producing bacteria; thereby improving CH_4 production. Also, the addition of these chelating ligands to the CM CD and CH digester systems reduced the HRT, from 50 days to 25 days, 52 days to 30 days and 57 days to 32 days in the CM CD and CH digester systems respectively. The decrease in HRT was due to the metal chelate catalysis of the hydrolysis stage of the anaerobic digestion process where the metal chelate formation served as the driving force in the solvolysis process. The effect of increasing chelating ligand concentration on production of H_2S was also studied. Also, the results showed a general decrease in H_2S concentration from 80 % to 30 % on

increasing the chelating ligand concentration within the best three performing substrate digesters namely, CM, CD and CH, from 10 μM to 100 μM. This reduction was due to the redox reaction occurring between the various metal chelates and H_2S, leading to the formation of elemental Sulphur with the release of H^+ ions; thus reducing the concentration of H_2S within the digester systems. Generally, addition of ligands to the substrate digesters increased production of CH_4, reduced HRT and decreased H_2S production greatly.

CHAPTER ONE

1.0 INTRODUCTION

Reliance on fossil fuels, also, the rate of energy demand in our world today far exceeds the rate of local supply and is causing a lot of apprehension since the sources of our irreplaceable fossil fuels are fast diminishing. Therefore, an important precautionary measure should be to develop renewable energy sources. These alternative sources of energy will take into consideration the present and future consumption of energy, minimize global warming, and improve national energy securities.

Unlike fossil fuels, biogas is bio-based and can be produced by organic fermentation of organic waste materials by the action of methanogenic bacteria (Dieter and Angelika, 2008). Depending on the substrate, biogas is principally a mixture of methane, carbon (IV) oxide and traces of hydrogen sulphide and ammonia (Awosolu, 2007). From a volumetric stand point, the trace gases (H_2S and NH_3) can be regarded as negligible. Biogas is renewable and an environmentally friendly alternative energy source as compared to non-renewable fossil fuels because it burns with minimal release of CO_2, and water (Teodorita *et al*., 2008). The emergence of biogas is currently engaging public interest as supplement to oil-base to serve as transportation or automobile fuels and also sources of heat and electricity generation (Dieter and Angelika, 2008).

1.1 Statement of Problem

A significant problem in the production of biogas is the prolonged hydraulic retention time (HRT). In a typical batch-type facility, the most probable retention time will vary from 50-80 days with an optimum operating temperature

of about 36^{o}C. But if the biogas production efficiency improves, this long HRT can be reduced appreciably leading to an equal reduction in industrial production cost. Also, the percentage composition of methane in biogas, which is the main constituent of fuel value, affects its calorific value. Natural gas consists of 91 % methane, 5.1 % ethane, 4.8 % propane, 0.9 % butane, and about 0.61 % CO_2.; while biogas consists mainly of 50-60 % CH_4 and 30-45 % CO_2 (Buratti *et al*., 2005). Based on the percentage of methane contained in it, natural gas has a higher calorific value of about 36.14 MJ/m^3 compared with biogas which has a calorific value of 21.48 MJ/m^3 (Dieter and Angelika, 2008). Therefore, improved percentage yield of CH_4 will increase biogas efficiency to a reasonable extent.

Another important factor that affects the efficiency of biogas is the presence of H_2S. H_2S is a highly toxic and corrosive gas. It is found in biogas at a concentration between 0-6 percent depending on the type of waste. H_2S is a major pollutant in fossil fuel based industries, sulphur based chemical industries and biogas based units (Kenneth *et al*., 2001). Improvement of biogas quality is essential because of the toxicity of this gas which affects the environment and its highly corrosive nature which causes corrosion of the metallic parts like boiler tubes engines coming in contact with it in a few hundred hours of operation. Internal Combustion (IC) engine application requires H_2S concentration to be less than 1000 ppm for a better engine life (Gary, 2003). Therefore, an appreciable increase in the percentage yield of CH_4 in biogas requires highly efficient methane producing digestion system with a low percentage of H_2S.

1.2 Fuels and their Characteristics

Fuel is any material that is capable of releasing energy when its chemical or physical structure is altered (Brian, 2000). Fuel releases its energy either through chemical means, such as burning, or nuclear means, such as nuclear fission or nuclear fusion (Angela *et al*., 2012). To utilize the energy of fuel in most usable form, it is required to transform the fuel from its chemical energy to some other form of energy via single or many stages. In this way, the energy of fuels can be utilized more effectively and efficiently for various purposes. There are various sources of fuels, they include:

1.2.1 Fossil Fuels

Fossil fuels are non-renewable energy sources that formed more than 300 million years ago during the carboniferous period (Brian, 2000). Fossils fuels are made from decomposed plants and animal matter. When plants and animals died, their bodies decompose; millions of years later, we have three forms of fossil fuel: oil, natural gas and coal (Heeter, 2006).

There are a number of problems associated with fossil fuels, most of which stem from the by – products created when they are burned to create energy. Chief among the by – products are CO_2 and N_2O, green house gases that are major contributors to global warming. Other by products of fossil fuels combustion include SO_2 and NO_2, both of which contribute to acid rain, and hydrocarbons, which react with NO_2 to form smog (Heeter, 2006).

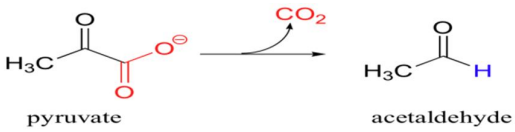
$$NO_2\,(g) + O_2\,(g) + \underset{\text{(unburnt petrol)}}{\text{Hydrocarbons}} \xrightarrow{\text{Sunlight}} \underset{\text{(Photochemical smog)}}{CH_3CO\text{-}OO\text{-}NO_2\,(g)} \qquad 1.1$$

In addition to their environmental harm, fossil fuels are limited resources that cannot be replaced (Murray, 2006), which is one major problem in our today's economy.

1.2.2 Solar Fuels:

Solar fuel is a fuel produced from sunlight through artificial photosynthesis or a thermochemical reaction (Angela *et al*., 2012; Matthew, 2013). Light is used as an energy source, with solar energy being transduced to chemical energy, typically by reducing protons to hydrogen, or CO_2 to organic compounds and/or CO (Matthew, 2013).

CO_2 + H_2O + Solar energy → O_2 +



pyruvate acetaldehyde 1.2

A solar fuel can be produced and stored for latter usage, when sunlight is not available, making it an alternative to fossil fuels. Solar fuels can be produced via direct or indirect processes. Direct processes harness the energy in sunlight to produce a fuel without intermediary energy conversions. This entails the use of catalyst that reduces protons to molecular hydrogen upon electrons from an excited photosensitizer. Their simplicity gives the advantage of potentially lower cost and increased energy conversion though this method has not yet been scaled up for commercial use (Andreiadis *et al.,* 2011).

In contrast, indirect processes have solar energy converted to another form of energy first (such as biomass or electricity) that can then be used to produce fuel. Hydrogen can be produced through electrolysis. To use sunlight in this

process, a photo-electrochemical cell can be used, where one photosensitized electrode converts light into an electric current that is then used for water splitting. This is an indirect process, since it produces electricity that is then used to form hydrogen (Styring, 2011). The other major indirect process is using photosynthetic organisms; however, most of the energy harvested in photosynthesis is used in life sustaining processes and therefore lost for energy use (Styring, 2011).

Indirect processes have been easier to implement but have the disadvantage of being less efficient since energy is wasted in the intermediary conversion for example splitting of water for the production of hydrogen (Styring, 2011). Another disadvantage of the use of solar fuels is the high cost of the equipment used to harness solar energy. Solar panels still remain a costly alternative to the use of readily available fossil fuel technologies. Also, solar panels require reliable quantities of sunshine to work efficiently. This means that the country of residence should be situated in an ideal part of the world that receives adequate sunshine.

1.2.3 Biofuels

Biofuels are renewable liquid fuels derived from microorganisms, plant or animal material (biomass), Fletcher *et al.,* (2011). They can be used, in their pure form or as part of blends, to fuel transport vehicles as well as to generate heat and power. With increasing concern about climate change, energy security and agricultural and economic development, interest in biofuels has grown rapidly in recent years (Fletcher *et al.,* 2011). The main types of biofuels

currently in commercial use are often referred to as first generation biofuels; they include:

i. **Bio ethanol:**

This is produced from sugar / starch from crops such as sugar cane, sugar beet, corn or wheat. Cellulostic biomass, derived from non-food sources, such as trees and grasses, is also being developed as a feedstock for bio ethanol production. Bio ethanol can be used as a fuel for vehicles in its pure form, but it is usually used as a gasoline additive to increase octane rating and improve vehicle emissions (Field *et al.,* 2008).

ii. **Bio diesel:**

Biodiesel can be made from oils of plants, for example, soya bean, oilseed rape, jatropha, sunflower or palm. Bio diesel is used as fuel for vehicles in its pure form, but it is usually used as a diesel additive to reduce levels of particulates, carbon (II) oxide and hydrocarbons from diesel-powered vehicles (Weiland, 2010).

iii. **Biogas:**

Biogas can be recovered from mechanical biological treatment waste processing systems (note that landfill gas, a less clean form of biogas, is produced in landfills through naturally occurring anaerobic digestion). It is a methane rich gas that can be compressed to fuel vehicles, and it is generated by anaerobic digestion of biomass.

1.3 Anaerobic Digestion

Anaerobic digestion is a biological process which can be used to convert in-expensive, readily available and inexhaustible resources, notably sewage sludge,

industrial and municipal waste and biomass material to valuable product gases, principally CH_4 (Marvin,1989). The process of anaerobic digestion has many advantages which include waste reduction, mitigation of global warming and most importantly, a source for the production of clean renewable energy. The biomass suitable for fermentation during the process of anaerobic digestion is named "substrate'', and the apparatus employed in carrying out the anaerobic digestion process is referred to as reactors or digesters.

1.4 Substrates for Anaerobic Digestion

Although all biomass containing carbohydrates, proteins, fats, celluloses, and hemicelluloses can be used as substrates, certain factors however need to be taken in to consideration when selecting the biomass:

i. the amount of the organic substance and
ii. the nutritional value of the organic substance.

These two points go a long way in affecting the final selection of biogas produced and smooth running of the fermentation process. If the substrate for anaerobic digestion is a combination of two or more feedstock types, the process is called "co-digestion". The co-digestion process is used to control the Carbon/Nitrogen (C/N) ratio. The process benefits of co-digestion are an improved rheological substrate quality and increased nutrient balance; also the process improves the methane yield because of the bacterial diversities in the different substrates, the positive synergies of established media and the supply of missing nutrients by co-substrates (Naomichi and Yutaka, 2007). Table 1.1 gives a list of the most common substrates categories used in the production of biogas.

Table 1.1 Types of Waste Materials Suitable for Biogas.

Sector	Production (tons/yr)	Type of waste
Pig	491500	Waste water with high organic content. mostly from manure Slaughterhouse waste water
Cattle/dairy	1720000	Waste water with high organic content, mostly from manure Waste water from slaughters/tamely processes with solids Waste water from dairy.
Poultry	17000000	Poultry manure Waste water from slaughters processes with solids
Mango	32000	Water from the process and thermal stage
Melon	190900	Water from the process and thermal stage (low concentration)
Citrus	459200	Husk/pulp waste Water from the process
Pineapple	950400	Husk waste and biomass, water from the
Banana	1739000	Water from the process in the packaging Empty fruit bunches waste
Coffee	846000	Waste water Pulp waste
African palm oil	666000	Waste water Nuts, pulp and empty fruit brunches
Sugarcane processing	3472000	Waste water Bagasse
Rice	211600	Husk
Industrial round wood	1687000 m^3/yr	Husk
Wood fuel	3463000 m^3/yr	Husk
Sawn wood	812000 m^3/yr	Sawdust and barks
Wood based panels	65 000 m^3/yr	Sawdust
Wood and fibre pulp	11 Mt/yr	Sawdust and barks
Paper and paper board	20 Mt/yr	Sawdust and barks
Landfills	4320000	Organic waste 58 %, paper 19 %, others 5 %
Domestic waste water	246000 m^3/yr	Domestic waste water into sewage

Source: FAO Statistics 2004 (www.fao.org/forestry).

1.5 Justification for the Study

i. The main raw materials used are basically waste products.

ii. The chelating ligands under study have been used at very low concentrations.

iii. Proper management of waste generated has been a cause for concern in major cities in both developed and developing countries.

iv. The anaerobic digestion is a treatment process used to solve multifaceted waste problems with a direct impact of reduction of green house gases and global warming.

v. The use of biogas as an energy source is fast replacing fossil fuels.

vi. The biogas technology can be used to improve rural infrastructure and to substitute imports and fossil energy sources.

1.6 Aim and Objectives

Based on the need for abundance or prolific supply of fuel, this research will focus on the study of the effects of chemical additives on solid waste digestion for the purpose of improving methane gas potential, reduction of hydrogen sulphide content and HRT of these waste samples via increased microorganism population in the waste.

The specific objectives of this work include:

i. optimization of the performance of the different chelating ligands and weights of substrates at which maximum methane gas production will be obtained.

ii. evaluating the effects of chelating ligands on the value of total volatile fatty acids and pH within the digester.

iii. analysis of substrates for percentage of total solids (% TS); volatile solids (%VS), carbohydrate content, total nitrogen content, moisture content and ash content.

iv. determination of trace metals present within the various waste samples.

v. investigation of the effects of the various optimized chelating ligands performance on increased concentrations, the hydraulic retention time, methane gas potential and hydrogen sulphide concentration.

vi. analyzing the effect of co-digestion on retention time, methane gas yield and hydrogen sulphide concentration.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HISTORY AND IMPORTANCE OF BIOGAS

There are historical evidence to show that the anaerobic digestion process is one of the oldest technologies. However, the industrialization of the anaerobic digestion process first began in India in the year 1859. In 1895, biogas was recovered from a sewage treatment facility and used to fuel street lamps in Exeter, England (Fraser, 1977).

Fraser, (1977) reported the identification of anaerobic bacteria and the conditions that promote methane production. As understanding of the anaerobic digestion process improved, more sophisticated equipment and operational techniques emerged; this resulted in the use of closed tanks, heating and mixing systems to optimize the anaerobic digestion process.

Regardless of these improvements, the anaerobic digestion process development suffered a great setback due to the emergence of coal and petroleum such that in developed countries the anaerobic digestion process was restricted to the treatment of waste water sludge. But in developing countries such as India and China, small scale anaerobic digestion systems strived but, were mostly for energy generation and sanitation purposes (Fraser, 1977). Nevertheless, the increase in energy prices have led to diversification of the waste treated and larger sized anaerobic digestion plants (Foresti, 2001).

In recent times, European countries have come under pressure to explore the anaerobic digestion market for two reasons: higher energy prices and

increasingly stringent environmental regulations. Record has it that the anaerobic facilities have been successful in treating a wide spectrum of waste streams such as municipal, agricultural or industrial wastes (Foresti, 2001). Now (Batstone *et al*., 2002), more than 600 farm-scale digesters operate in Europe, where the key factor is simplicity. In addition to farm-scale digesters, Europe leads the world in large centralized anaerobic digestion systems.

The anaerobic digestion process has been investigated extensively during the past decades because of its importance in converting inexpensive, readily available and inexhaustible resources, notably sewage sludge, industrial and municipal waste and biomass material to valuable product gases, principally methane. The importance of the anaerobic digestion process and biogas production includes:

2.1.1 Direct Combustion.

The most common application of biogas is the direct combustion in natural gas burners. Biogas can be burned for heat production either on site or transported by pipeline to the end users. For heating purposes, biogas does not need any form of upgrading, and the contamination level does not restrict the gas utilization as much as in the case of other applications like automobile fuels (Gunuseelan, 1997). However, biogas needs to undergo condensation and particulate removal, compression, cooling and drying (Prasad *et al*., 2008).

2.1.2 Combined Heat and Power (CHP) Generation.

In many countries with a developed biogas sector, CHP generation is considered a very efficient utilisation of biogas for energy production. But before use, the biogas is usually drained and dried, because most engines have maximum

tolerance limit for the content of hydrogen sulphide and halogenated hydrocarbons (Karagiannidis and Perkoulidis, 2009). An engine base CHP plant has an efficiency of up to 90 % and produces 35 % electricity and 65 % heat (Jha *et al*., 2010). The most common types of CHP are the Block Type Thermal Power Plant (BTTP) with Gas-Otto, or gas-pilot injections engines. Most of these BTTP motors have engines which require biogas with a minimum methane content of 45 %. Another major advantage of these engines is that they emit less carbon (II) oxide (Pind *et al*., 2003a).

2.1.3 Fuel Cells Production.

Fuel cells are electrochemical devices that convert the chemical energy of a reaction directly into electrical energy (Naomichi and Yutaka, 2007). The basic building block of a fuel cell consists of an electrolyte layer in contact with a porous anode and cathode on both sides. In a typical fuel cell, the anode could be fed continuously by waste materials (biomass) used for generating the biogas while an oxidant is fed continuously to the cathode compartment. The presence of micro-organisms e.g. *Shewanella putrefaciens* and *Aeromonas hydrophila* within the biomass help to degrade the carbohydrate into glucose and also serve to transfer electrons directly to the electrode without the need of mediators. An electrochemical reaction takes place at the electrodes and electric current is generated (Oliveira and Rosa, 2003). See Figure 2.1.

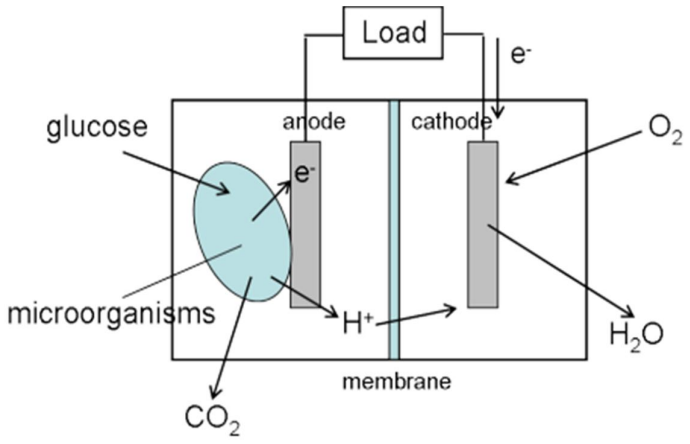


Figure 2.1: Schematic diagram of a simple biogas fuel cell (Willy, 2010).

2.1.4 Biogas as Automobile Fuel.

Utilization of biogas in the transport sector is a technology with great potential and with important socio-economic benefits. Biogas is already being used as automobile fuel in countries like Sweden, Germany and Switzerland. The number of private cars, public transportation vehicles and trucks powered by biogas (mainly methane gas) is increasing. In many European countries, diesel buses are being replaced with methane powered ones (Schulte and Kottwitz, 1982). Heavy duty vehicles can equally be converted to run as methane gas only. Methane run vehicles have a lot of advantages over the engines operating on fossil fuels; the over-all advantage being the drastic reduction in emission of carbon (IV) oxide (Schulte and Kottwitz, 1982). Emission of particles and soot are also drastically reduced when compared with the very modern diesel engines, equipped with particle filters. Also, upgraded biogas (bio-methane) is

considered to have the highest potential as automobile fuel even when compared to other bio-fuels like bio-ethanol and bio-diesel (Edelmann, 2003).

2.2 Types of Anaerobic Digestion Systems.

Anaerobic digesters can be classified into the following categories:

i. the batch systems;
ii. the continuously stirred systems; and
iii. the two-stage systems.

2.2.1 The Batch Systems.

In the batch systems, digesters are filled once with fresh water and allowed to undergo the process of degradation sequentially. A minimum amount of water is usually added and leachate collected at the bottom of the fermenting waste recirculated to the top (Brummeler, 2000). There are two types of batch systems:

i. Single Stage System.

Here, the system involves a simple recirculation of the leachate at the top of the same reactor, which is equivalent to a partial mixing.

ii. Sequential System.

This system involves two or more digesters. The leachate of a freshly filled reactor, containing high levels of soluble organic compounds, is recirculated to another, more mature digester where methanogenesis takes place. The leachate of the latter digester, containing little acids and loaded with pH-buffering bicarbonates, is then pumped back into the initial digester. This configuration guarantees inoculation between the two reactors, which eliminates the need to

mix the fresh water with seed materials (Brummeler, 2000); (Lissens *et al*., 2001).

The advantage of the batch systems is that, it is simple, inexpensive, useful in laboratory-scale studies in accessing the rate at which a material can be digested and determining the yields of biogas obtainable. (Stewart *et al*., 1984; Santosh *et al*., 1999; Lettinga, 2001; Lissens *et al*., 2001; Lastella *et al*., 2002). The major disadvantage of batch systems is the lower biogas yield caused by clogging due to the compaction process (Ouedraogo, 1999).

2.2.2 The Continuously Stirred Systems.

For this system, the digester is fed intermittently; the most common frequency being once a day, at a loading rate of 1.7kg (Ouedraogo, 1999). The digester is continuously mixed, and the mixing may be achieved by mechanical means or by gas recirculation, where digester gas is bubbled back through the digester contents (Hawkes and Hawkes, 1987). The main disadvantage with this process is that the effluent will contain some fraction of freshly added, undigested feed material, together with some of the still active microorganism and the CH_4 yield is usually low. The system also requires large amounts of water which leads to high cost for treatment of substrate (Gunaseelan, 1997).

2.2.3 Two-Stage Systems.

The two-stage system which is sometimes referred to as the multi-stage anaerobic digestion, is a process involving the use of two separate reactors, one for liquidation (hydrolysis) and acidification, and the other for acetogenesis and methanogenesis, connected in series. The development of the multi-stage

systems is aimed at improving the anaerobic digestion process by having separate digesters per the acid and CH_4 forming population's thus providing flexibility to optimize each of these reactions (Pohland and Ghosh, 1971; Callaunder and Barford, 1983; Cohen *et al*., 1982). In the first reactor, the reaction is limited by the rate of hydrolysis, while the second, by the rate of microbial growth (Ghosh and Pohland, 1974; Vavilin *et al*., 2001). The two-stage system allows for optimisation of both microbial processes with respect to nutritional requirements, physiology, physical requirements and growth rates (Anderson *et al*., 1994; Elefsiniotis and Oldham, 1994; Ghosh *et al*., 2000). The acidogenic-stage digester is usually maintained at a low alkalinity and develops a high CO_2 and low CH_4 content in the gaseous environment. The major biochemical reaction occurring at this stage is hydrolysis and fermentation. The second digester is maintained at a pH above 7 (high alkalinity) resulting in high specific methanogenic activity (Ghosh, 1990; Fox and Pohland, 1994). This two-stage system of anaerobic digestion has the major advantage of higher CH_4 concentration (80-85 %) in the biogas produced because the specific activity of methanogenesis is increased.

2.3 The Processes involved in Anaerobic Digestion.

The conversion of organic matter to methane and carbon (IV) oxide is presently thought to occur in four stages each of which involves a different type of bacteria population. These stages involve the following:

I. Hydrolysis:

During hydrolysis, polymers like carbohydrates, lipids, nucleic acids and proteins are converted into glucose, glycerol, purines and pyrimidines. This

process is carried out by the hydrolytic bacteria (e.g. *Clostridium* and *Bacteroides*). These bacteria excrete hydrolytic enzymes, which convert biopolymers into simple soluble compounds as shown below;

$$\text{Lipids} \xrightarrow{\text{lipase}} \text{fatty acids, glycerol} \qquad 2.1$$

$$\text{Polysaccharide} \xrightarrow{\text{cellulose, xylanese,amylase}} \text{monosaccharide} \qquad 2.2$$

$$\text{Nucleic acids} \xrightarrow{\text{glycine reductase}} \text{purines, pyrimidines} \qquad 2.3$$

$$\text{Proteins} \xrightarrow{\text{protease}} \text{amino acids} \qquad 2.4$$

II. Acidogenesis:

This involves the degradation of simple sugars and amino acids, to carbon (IV) oxide and hydrogen as well as into volatile fatty acids (VFA) and alcohols. For example, in the degradation of carbohydrates, pyruvates which are the end products of the glycolysis of glucose are degraded by a series of steps to form propionic acid (Figure 2.2). These components (e.g. simple sugars and amino acids), are degraded by a group of bacteria called the acidogenic bacteria (e.g. *Clostridium* and *Bacteroides*). This process leads to the third stage known as acetogenesis.

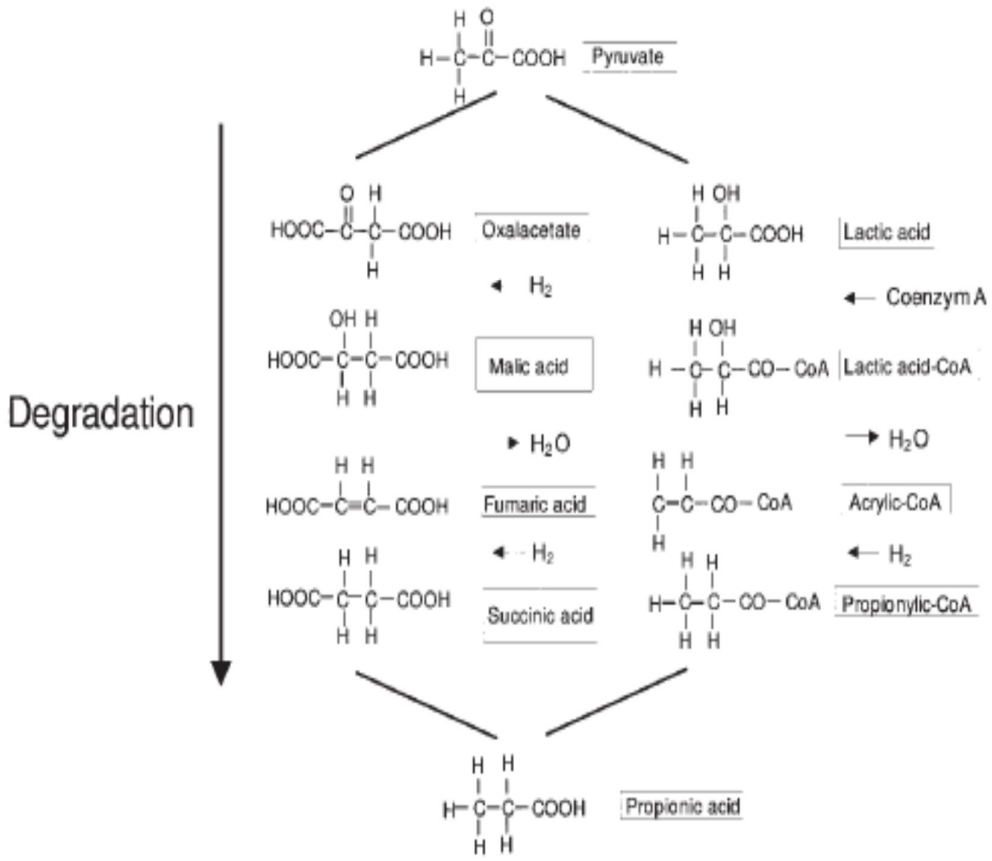


Figure 2.2: Degradation of Pyruvate (end product of glucose degradation) to Propionic acid (AL Seadi, 2001).

III. Acetogenesis:

At this stage products from acidogenesis which cannot be directly converted to methane by methanogenic bacteria are converted into methanogenic substrates. VFA and alcohols are oxidised into methanogenic substrates like acetate, hydrogen and carbon (IV) oxide. This degradation is carried out by the acetogenic bacteria (*Syntrophobacter wolinii* and *Syntrophomonas wolfei*). The production of hydrogen increases the hydrogen partial pressure. This can be regarded as a waste product of this stage that inhibits the metabolism of the acetogenic bacteria.

IV. Methanogenesis:

This final stage of anaerobic digestion involves the methanogenic bacteria (*Methanosarcina spp*. and *Methanothrix spp*.). Some of these bacteria convert acetic acid to CH_4 while others utilize the hydrogen and carbon (IV) oxide produced to form CH_4. According to the following equations;

$$\text{Acetic acid} \xrightarrow{\text{methanogenic bacteria}} \text{methane + carbon (IV) oxide} \qquad 2.5$$

$$\text{Hydrogen + carbon (IV) oxide} \xrightarrow{\text{methanogenic bacteria}} \text{methane + water} \qquad 2.6$$

Depending on the composition of the substrate fed into an anaerobic digester, different stages may-be the rate-limiting step in the digestion process. In the digestion of soluble compounds, the final stage has been identified as the rate limiting step. While for insoluble compounds, the rate limiting step is the hydrolysis of these compounds (Al Seadi, 2001).

2.4 Factors Affecting Anaerobic Digestion.

Since the process of anaerobic degradation is carried out by a variety of microorganisms which are greatly influenced by some critical parameters, thus it becomes imperative that the optimum conditions of operation for the anaerobic microorganisms be provided. These conditions include:

(i) temperature, (ii) pH-value, (iii) available nutrients and (iv) presence of inhibitors.

2.4.1 Temperature

The temperature at which a digester functions has a critical impact on the biogas production process. The digestion process can be performed in one of the three

ranges of the temperature: Psychrophilic (below 25^0C), mesophilic (25^0C-45^0C), and the thermophilic (45^0C-70^0 C). There is a direct relationship between the process temperature and the HRT as shown in Table 2.1.

Table 2.1: Temperature and its Effect on Typical Retention Times.

Thermal Stage	Process Temperatures	Minimum Retention time
Psychrophilic	20 to 25^0C	70 to 80 days
Mesophilic	30 to 42^0C	30 to 40 days
Thermophilic	43 to 55^0C	15 to 20 days

(LfU, 2007)

Many modern biogas plants operate at thermophilic process temperatures especially as it allows for a reduced HRT, better degradation of solid substrates and higher growth rate of methanogenic bacteria (Bhattacharya and Mishra, 2003; Schafer *et al*., 2006). But there lies a major disadvantage in this thermophilic process which is the increase in the ammonia toxicity. High ammonia concentrations can be significantly toxic for anaerobic bacteria and can inhibit the production of methane (Schafer *et al*., 2006). Also, the viscosity of the anaerobic digester substrate is inversely proportional to temperature. This means that at higher temperatures, the diffusion of dissolved material is greatly improved due to the fact that the substrate is liquid at this stage. Thermophilic operational temperatures therefore results in faster chemical reaction rates better

efficiency of methane production, higher solubility and lower viscosity (Jha *et al*., 2010).

2.4.2 pH Values

pH plays an important role when considering the growth of microbial life during digestion. Research shows that methane formation takes place between the pH intervals of about 5.5 to 8.5 with an optimum interval between 7.0 to 8.0 (Lay *et al*., 1997). In anaerobic treatment processes, the pH is typically maintained at conditions more optimal for methanogens to prevent the predominance of acid-forming bacteria, which may cause the accumulation of VFAs, which is common at low pH-values (Fatma *et al*., 2009).

For mesophilic digesters, the optimum pH interval ranges from 6.5 - 8.0 and the process is severely limited if the pH-values decreases below 6.0 or rises above 8.5. Decrease in the pH value ranging from 3.5 - 5.0 is due to the accumulation of VFAs, while increase in pH values is due to increase in the production of ammonia (Ward *et al*., 2008). The value of pH in anaerobic reactors is mainly controlled by the bicarbonate buffer system. Therefore, the pH value inside digesters depends on the partial pressure of CO_2 and on the concentration of alkaline and acidic components in the liquid phase (Sandberg and Ahring, 1992). If accumulation of base or acid occurs, the buffer capacity counteracts these changes up to a certain level. When the buffer capacity is exceeded, a drastic change in the pH-values occurs completely inhibiting the anaerobic digestion system (Fatma *et al*., 2009).

2.4.3 Available Nutrients.

Trace elements such as iron, nickel, cobalt, selenium, manganese and zinc are equally important for the growth and survival of anaerobic microorganisms, as well as the macronutrients which include: carbon, nitrogen, phosphorus, and sulphur. The optimal ratio of the macronutrients carbon, nitrogen, phosphorus, and sulphur (C: N: P: S) is considered to be 600:15:5:1. However, each of these substances at high concentrations typically would have an inhibitory effect on the anaerobic digestion process (Lettinga, 2001).

2.4.4 Presence of Inhibitors

Ammonia (NH_3) is an important compound, with a significant function for the anaerobic digestion process. NH_3 is an important nutrient, serving as a precursor to foodstuffs and fertilizers and is normally encountered as a gas, with a characteristic pungent smell. Proteins are the main source of ammonia for the anaerobic digestion (AD) process. Too high ammonia concentration inside the digester, especially free ammonia (the unionised form of ammonia), is considered to be responsible for process inhibition (Kayhanian, 1999). This is common to AD of animal slurries, due to their high ammonia concentration, originating from urine. For its inhibitory effect, ammonia concentration should be kept below 80 mg/L. Methanogenic bacteria are especially sensitive to ammonia inhibition (Teodorita *et al*., 2008). The concentration of free ammonia is directly proportional to temperature, so there is an increased risk of ammonia inhibition of AD processes operated at thermophilic temperatures, compared to mesophilic ones. When a process is inhibited by ammonia, an increase in the concentration of VFA will lead to a decrease in pH. This will partly counteract

the effect of ammonia due to a decrease in the free ammonia concentration (Teodorita *et al*., 2008).

2.4.5 Volatile Fatty Acids (VFA)

VFAs are intermediate compounds. Examples include propanoic acid, acetic acid and butanoic acid. These compounds which are produced during the process of acidogenesis could accumulate inside the digester which could further reduce the pH. However, the accumulation of VFA is not always accessed by a drop of pH value due to the buffer capacity of the digester. This means that the VFA accumulation could exceed a certain level, before it can be detected by a drop in pH value. At such point, the VFA concentration in the digester would be so high that the anaerobic process will be already severely inhibited (Guendouz *et al*., 2010).

Practical experience shows that two different digesters can behave totally different with respect to the same VFA concentration, so that one and the same concentration of VFA can be optimal for one digester, but inhibitory for the other one (Viéitez *et al*., 2000). One of the possible explanations can be the fact that the composition of microorganism populations varies from one digester to the next (Hanaki *et al*., 1994). For this reason, and like in the case of pH, the VFA concentration cannot be recommended as a stand-alone process monitoring parameter.

2.4.6 Hydraulic Retention Time (HRT)

HRT is the average time interval when the substrate is kept inside the digester. The HRT is affected by the amount of organic load, temperature, and substrate composition within the digester system. A long HRT requires more substrate and invariably implies a higher cost of production. Whereas, a short HRT

provides a good substrate flow rate, it is usually associated with a lower gas yield which on its own is a major disadvantage (Nges and Liu 2010).

2.5 Advantages of the Biogas Technology.

There are a number of benefits to be derivable from the process of anaerobic digestion which affects the environment and socio-economy of countries where the technology has been employed. A number of these advantages include:

2.5.1 Mitigation of Green House Effect

The usage of fossil fuels such as lignite, hard coal, crude oil and natural gas has significantly increased in the last few decades. This in turn has led to a further increase of current CO_2 concentration in the atmosphere leading to global warming as CO_2 is a green house gas (Rittman and McCarty, 2001). The combustion of biogas also releases CO_2 but in very low concentrations (Braber, 1995). Also, the process of storage and utilization of animal manure as fertilizer has increased the amount of CH_4 gas which has a higher green house gas potential than CO_2. But with the utilization of these animal wastes in the production of biogas which could serve as a convenient replacement to fossil fuels, a significant reduction of emission of CO_2, CH_4 and even N_2O will occur; resulting in the mitigation of global warming (Edelmann, 2003).

2.5.2 Reduced Dependence on Imported Fuels

Most countries are strongly dependent on fossil fuel importation from regions rich in fossil fuel such as the Middle East and Russia. The development and implementation of renewable energy sources, such as biogas from anaerobic digestion systems will go a long way in reducing dependency of these countries

and also improving their national energy supply (Angelidaki and Saunders, 2004; Chen *et al*., 2008).

2.5.3 Waste Reduction

Increased industrialization has led to over-production of organic wastes from chemical, agricultural and even pharmaceutical industries (Lou and Nair 2009). A major advantage of the biogas technology is in its ability to transform these waste materials into a valuable resource by using it as a substrate for anaerobic digestion, followed by recycling of the digested substrate as fertilizer. The biogas technology has also contributed to a reduction in the volume of waste and cost of waste disposal (Chen *et al*., 2008).

2.5.4 Improved Income for Farmers

Production of feedstock in combination with operation of biogas plants makes biogas technology economically attractive for farmers and provides them with additional income. The farmers also get a new important social function as energy provider and waste treatment operator (Ahn and Smith, 2008).

2.5.5 Fertilizer

The digested substrate (digestate) which is a by-product from the biogas technology is a valuable soil fertilizer rich in nitrogen, phosphorus, potassium and micronutrients. Digestates has improved fertilizer efficiency when compared to raw animal manure (Kottner, 2005).

2.5.6 Job Creation

The biogas technology requires man power for production, collection and transportation of anaerobic digestion feedstock, manufacture of technical equipment, construction and operation of the biogas plant (Li *et al*., 2011). Therefore the development of a national biogas sector contributes significantly to a country's economic potential and creates job opportunities.

2.6 Principles of Analytical Techniques Employed in Biogas Generation

2.6.1 Principle of Elemental Determination using Atomic Absorption Spectrophotometer (AAS)

The solutions containing the metals of interest were aspirated into a suitable flame of a flame atomic absorption spectrophotometer (AAS) in which the thermal energy was used to decompose the compounds containing the metals setting free the metal atoms in the vapour phase. Majority of the atoms remained in the ground state. The atoms were then irradiated with radiation of characteristic wavelength from the hollow cathode lamp and atoms of particular elements absorbed radiation characteristic of the element, hence other elements in the same solution did not interfere. As the atoms absorbed this radiation, the decrease in intensity was measured by a monochromator and detector. The degree of absorption and the decrease in intensity of the radiation were then related to the concentrations of the elements in the solutions.

2.6.2 Principle of Biogas Characterization using Biogas 5000

The Biogas 5000 analyzer provides an accurate and consistent method of monitoring the composition of the biogas produced from each digester. It operates on the principle of infra-red (IR) absorption for the measurement of

CO_2 and CH_4. The radiation from a broadband IR source is passed through filters to select only the wavelengths that will be absorbed by CO_2 and CH_4. A gas sample is pumped from each digester into the measurement cell of the biogas analyzer where the IR radiation is passed through the gas onto a detector. The detector then identifies and measures each constituent gas present within the sample.

2.7 Techniques Employed for the Enhancement of Biogas

Despite its numerous advantages, the full potential of the biogas technology cannot be fully harnessed as certain constraints are associated with it. Most common among these are: the large hydraulic retention time of 30-80 days and low gas production in most digestion systems (Cho *et al*., 1995). Thus, to remove these limitations, researchers have tried different techniques some of which include: recycling of digested slurry, variation of operational parameters and the use of biological and/or chemical additives (Kobayashi et al., 1998).

2.7.1. Recycling of Digested Slurry

This technique has been shown to improve the biogas production marginally, since microbes washed away are reintroduced into the digester, thereby providing an additional microbial population. The recycling of the digested slurry along with filtrate has also been tried out to enhance biogas production (Malik and Dahiya, 1990). Research has shown that about 60-65 % more biogas can be produced by simply recycling the digested slurry (Kanwar and Guleri 1994) as well as maintaining higher gas production under psychrophilic temperature (Chugh *et al*., 1999).

This process is also useful in improving the rate of the digestion process. Malik and Tauro (1995), carried out an experiment where pre-digested slurry was used along with 10 % effluent slurry recycling in 1 dm^3 biogas digester. The HRT reduced from 50 days to 30 days with an 18.8 % increase in gas production. Although, the recycling of slurry can improve the process performance, it has a major disadvantage of poor start-up performance and incomplete mixing and accumulation of VFA (Amani *et al*., 2011).

2.7.2. Variation of Operational Parameters

a. Effect of Temperature on efficiency of Digestion process

The anaerobic digestion process can take place at psychrophilic, mesophilic or thermophilic temperatures. But most reactors operate optimally at temperatures in the mesophilic and thermophilic range (Umetsu *et al*., 1992; Takizawa *et al*., 1994 and Mital, 1996).

Compared with the mesophilic digesters, thermophilic digesters show more rapid degradation of waste (Hegde and Pullammanappallil, 2007). Moreso, the biodegradability and methane yield have been found to be greater at temperatures as high as 55°C (thermophilic) than at 35°C (mesophilic) (Lu *et al*., 2007). Also, Molnar and Bartha (1988), discovered that a gradual decrease in temperature from 55°C to below 35°C, resulted in decrease in gas production rate from 2.53 to 0.7 m^3/day in a 80 dm^3 batch digester. Though, thermophilic systems performed well over the mesophilic systems this was not without the associated problem of increased energy required for maintaining the digesters at the higher temperatures (Juanga, 2005; Novak, 2007), and also, the problem of

increased risk of ammonia formation which could invariably reduce the volume of biogas produced (Angelidaki and Sanders, 2004).

b) Effect of pH on Efficiency of Anaerobic Digestion process

The pH is an important principal operational parameter affecting the growth of microbes during anaerobic digestion. The desired pH for methanogens ranges from 6.8 to 7.6, and their growth rate will be greatly reduced if the pH drops to below 6.0 (Mosey and Fernandes, 1989). Ghaly (1996) discovered that a two-phase digester using cheese and dairy manure as substrate operated as a single-phase reactor when the pH was not controlled. However, when the pH was controlled in the methanogenic stage, it operated as a two-stage two-phase digester with remarkable improvement in methane production. The major problem associated with use of pH as the sole parameter in improving biogas yield is the difficulty encountered in ensuring that the pH is kept within the optimum range.

c) Effect of Pre-treatment of Substrate

Feed-stocks sometimes require pre-treatment in order to increase the percentage of methane produced in the process of anaerobic digestion. The hydrolysis stage (i.e. the biological degradation of cellulosic organic matter), is the principal rate determining step which is the target in pre-treatment processes.

Pre-treatment could be carried in any of the following ways:

i. Pre-treating of substrate with alkali or acid

ii. Pre-digestion of fresh substrate

iii. Thermo chemical pre-treatment

iv. Ultrasonic pre-treatment

v. Ensilage of substrate

The primary objective of all pre-treatments regardless of the method is the destruction of sludge flocs and the rupturing of cell walls to release cell components so that they are accessible for chemical and biological processes (Muller, 2001).

Research has shown that the thermo-chemical pre-treatment of water hyacinth greatly improved the process of bio-methanation with the best results at a pH of 11.0 and a temperature of 21°C (Patel *et al*., 1992). Ultrasonic pre-treatment of waste activated sludge for 30 minutes has equally resulted in a 64 % increase in methane production (Wang *et al*., 1999). Also, Dar and Tandon (1987), observed an improvement of 31- 42 % in microbial digestibility and an almost twofold increase in biogas when alkali treated plant residues were used as supplement to cattle dung.

Although, pre-treatment methods have been shown to deliver optimal results for a number of objectives, however, it should be noted that the objectives of pre-treatment need to be clearly defined before a technology is selected, since a treatment to improve, say biodegradability, may have negative impact on another treatment stage, for example dewatering (Kopp *et al*., 1997). Another potential downside to the use of pre-treatment method is the potential disruption of the digestion process by the dissociation of methanogenic and acetogenic bacteria such that their symbiotic degradation of sludge solids is disturbed (Muller *et al*., 2004).

d) Effect of Agitation and Particle Size on Anaerobic Digestion Efficiency

Although agitation and particle size are not very important parameters as pH or temperature of the digester contents, they still have some measure of effect on biogas production. For instance, smaller particle sizes would provide larger surface area for adsorbing the substrate, which will in turn result in increased microbial activity and hence increase biogas production (Sharma *et al*., 1988). Also, the use of agitation will ensure intimate contact between microorganisms and substrate leading to improved digestion processes and thus enhance biogas production (Baier and Schmidheiny, 1997).

2.7.3. Use of Biological and/or Chemical Additives

Biological additives such as crop residues, plants, weeds, and microbial cultures, are available naturally in the surroundings with less significance in terms of their use in their natural habitat. However, if harnessed as additives in biogas plant could improve its performance significantly (Gunaseelan, 1987). The biological and chemical additives are believed to increase biogas production by stimulating the microbial activity within digester systems (Attar *et al*., 1998).

i) Microbial Strains

Cellulolytic strains of bacteria like *actinomycetes* have been found to improve biogas production in the range of 8.4 – 44.4 % from cattle dung (Tirumule and Nand, 1994; Attar *et al*., 1998). Geeta *et al*., (1994), found that sugar cane bagasse pre-treated with *Phanerochaete chrysosporium* for three weeks under ambient temperature conditions produced higher gas with cattle excreta. Also, microorganism producing hydrolytic enzymes have been proven to improve the rate of hydrolysis reaction by causing the

solubilisation of extracellular polymeric substance in activated sludge thereby increasing biogas production by 60 % (Wawrzynczyk *et al*., 2003).

ii) Addition of Minerals

Uemura (2010), observed that the addition of minerals (Ni, Co and Fe) in the batch mesophilic digesters treating organic municipal waste improved the digester performance and biogas yield. More so, the additives also helped to maintain favourable conditions such as pH and promotion of methanogenesis within the digester for rapid biogas production. Dhawale (1996), also found 25-35 % enhancement in anaerobic digestion of manure by the addition of Eosin blue dye at 0.1 μM concentration. The use of additives is fast becoming the focus of recent research efforts into the optimization of CH_4 gas produced from smaller digesters (Song *et al*., 2012). The effects of different concentration of NaOH were subsequently investigated. Results indicated that the rate of (Chemical Oxygen Demand) COD removal increased as the NaOH dosage increased with a maximum value of 43.5% when 7g/L NaOH were added. Above this concentration, a lower rate of COD solubilisation was observed. Subsequent digestion of sludge treated with 7g/L NaOH for 7 days at 37 ^{o}C resulted in volatile solid (VS) reduction in the pre-treated sludge of 29.8 % compared the 20.5 % of the control while methane and biogas production increases of 12.7 % and 13.3 %, respectively were observed (Jeongsik *et al*., 2003).

Trace metals (Fe, Co, and Ni) are considered as the most essential nutrients for microbial uptake and growth (Marvin, 1989). A partial list of chelating ligands and their log stability constants is given in Table 2.2. The affinity is generally greatest for Fe. The uptake of these trace metals within the various

substrates depends on the metal speciation and bioavailability. The speciation is controlled by metal concentration, digester operational conditions as well as chemical processes such as complexation (Mosey *et al.,* 1971 and Pinheiro and van Leeuwen, 2001). It is also assumed that these metals are only available for microbial uptake when they are present as free metal ions and as metal complexes in the soluble form (Saito *et al.,* 2002; Worm *et al.,* 2006). Since most of these metals are present as metal sulphides within the digester systems (Rinzema and Lettinga, 1988); therefore the presence of organic and inorganic chelates will affect the metal bioavailability by the formation of metal chelates with high stability constants. Note that the larger the stability constant, the higher the proportion of the chelates that exists in solution and thus, the greater its availability to the methanogens (Marvin, 1989). Therefore, it is believed that stimulation of the anaerobic digestion process results from the degree of binding of Fe Co and Ni to chelating ligands in the various substrates. This leads to an increase in the solubility of these essential inorganic nutrients which in turn promotes the growth and stability of the methanogenic population and thereby improving CH_4 production.

Faster start-up, greater stability and more rapid recovery from upsets are possible by the use of this new method i.e. addition of chelating ligands.

Table 2.2: Logarithm Stability Constants of Some Chelating Ligands

Chelating Ligand	Fe^{3+}	Co^{2+}	Ni^{2+}
EDDA	8.63	11.25	13.6
NTA	15.87	10.38	11.54
DTPA	28.60	18.40	20.32
EDTA	25.10	16.31	18.62
PORPH	7.0	2.9	3.8
MESYL	30.6	-	-
PENI	-	-	-

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Samples Collection

The substrate used in this work include: Guinea corn chaff (GU), Barley chaff (BL), Cow manure (CM), Chicken droppings (CH), and Cow cud (CD). These were obtained from different industrial sites within Kaduna State as shown in Table 3.1

Table 3.1: Industrial Sites from where Each Sample was sourced

Substrate	Areas of Location
GU	International Beer and Beverage Industries Nigeria Limited.
BL	International Beer and Beverage Industries Nigeria Limited.
CM	Anguwan-Yusi, Hanwa N/Extension, Zaria
CD	Zaria Abattoir
CH	National Animal Production Research Institute, Zaria

3.1.2 Substrate Preparation

All substrates after collection were stored in an air tight polythene bags and transferred to the Department of Chemistry Laboratory for further processing. The various waste samples obtained from different sources were transported to the laboratory where they were sun-dried for 72hours; then ground with a mortar and pestle to smaller particle sizes of about 30.0mm.

The ground samples were then properly labelled and stored in air-tight plastic containers until when needed.

3.1.3 Apparatus/Equipment

i) Biogas Analyzer (Biogas 5000, UK)

ii) An Atomic Absorption Spectrophotometer (UNICAM 929, UK) with an absorbance range from 0 to 1.999.

iii) A pH meter (Jenway 3505, UK) with a pH range of -2 to 16 ± 0.001.

iv) A Muffle Furnace (Carbolite, UK), with a temperature between 0 to 1100°C.

v) A Magnetic Stirrer Hotplate (Jenway1000, UK).

vi) An Analytical Digital Balance accurate to 0.1 mg. (Sartorius, USA)

vii) Desiccators of different sizes

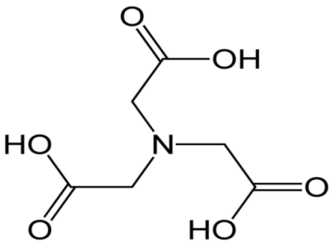
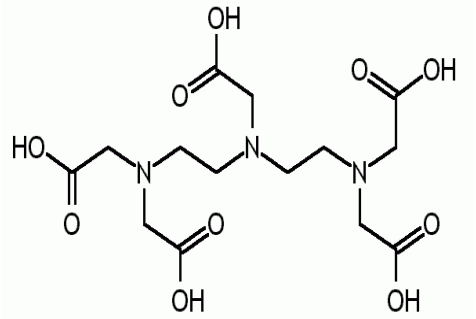
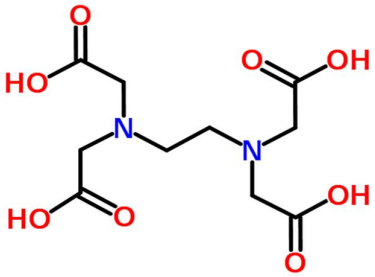
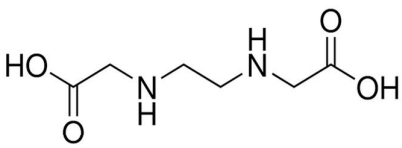
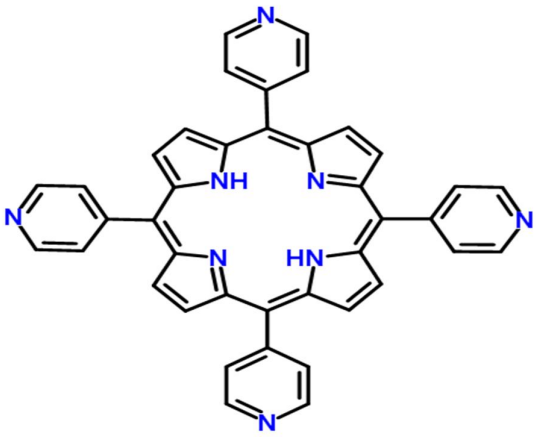
viii) An Oven (Gallenkamp, Germany) with temperature range between 0 to 200°C was used throughout the study.

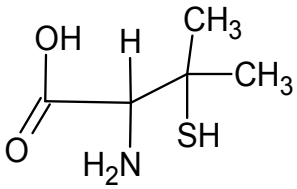
ix) Mortar and Pestle

3.1.4 Reagents and their Preparations

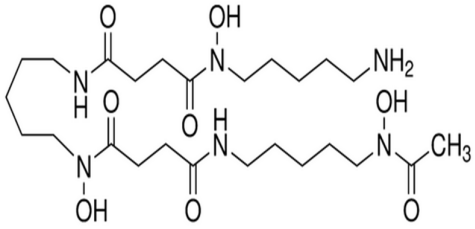
All reagents used were of analytical grade. Deionized water was used in preparation of all aqueous solutions; all prepared solutions were stored in amber coloured bottles. Table 3.2 gives a list of the chelating ligands used and their names.

Table 3.2: Structures and Names of the Chelating Ligands

CHELATING LIGAND	NAME
	Nitrilotri-acetic acid (NTA)
	Diethylenetriamine-pentaacetic acid (DTPA)
	Ethylene diamine-tetraacetic acid (EDTA)
	Ethylene diamine-N, N-diacetic acid (EDDA)
	5,10,15,20-tetra(4-pyridyl)-21H,23H- Porphine (PORPH)



D-Penicillamine (PENI)



Deferoxamine mesylate (MESYL)

i) Sodium Hydroxide Solution (40%).

Forty percent sodium hydroxide solution was prepared by dissolving 40 g sodium hydroxide pellets in deionized water in a 100cm^3 volumetric flask. This was then made up to the mark with deionized water.

ii) Sulphuric Acid (0.05M)

Sulphuric acid (2.72 cm^3) was transferred using a 5 cm^3 measuring cylinder into a one-litre volumetric flask containing 800 cm^3 of deionized water. The solution was then made to the mark with deionized water.

iii) Normal Saline (0.9%)

Normal saline (0.9 %) was prepared by dissolving 9 g sodium chloride salt in 100 cm^3 of deionized water in a 1 litre volumetric flask. The solution was then made up to the mark with deionized water.

iv) NTA Stock Solution (1000 µM)

NTA salt (191 mg) was weighed and transferred into a 1000 cm^3 volumetric flask containing 500 cm^3 of deionized water. The mixture was continuously stirred with a glass rod till the salt completely dissolved; then made up to mark with deionized water.

v) EDTA Stock Solution (1000µM)

EDTA (292 mg) was weighed out and transferred into a 1litre volumetric flask. About 600 cm^3 of deionized water was added to the flask with periodical swirling till all the solids dissolved. The solution was then diluted to the mark with deionized water.

vi) EDDA Stock Solution (1000 µM)

EDDA salt (176.2 mg) was weighed out using an analytical digital balance (Satrorious, USA) into a 1000 cm^3 volumetric flask containing 600 cm^3 of deionized water. The mixture was stirred continuously with a glass rod till a solution was obtained which was diluted to the mark with deionized water.

vii) DTPA Stock Solution (1000 µM)

DTPA (393.4 mg) was weighed and transferred into a 1 litre volumetric flask. And about 700 cm^3 of deionized water was added with continuous stirring for at least 3 hours using a magnetic stirrer (Jenway 1000, UK) until the salt was fully dissolved. The solution was then diluted to the mark with deionized water.

viii) MESYL Stock Solution (1000 µM)

MESYL salt (656.8 mg) was weighed out into a 1000 cm^3 volumetric flask containing 500 cm^3 of deionized water. The mixture was continuously stirred with a glass rod till the salt completely dissolved; then made up to the mark with more deionized water.

ix) PENI Stock Solution (1000 µM)

PENI salt (149 mg) was weighed and transferred into a 1litre volumetric flask. 600 cm^3 of deionized water was added with a magnetic stirrer till the salt completely dissolved. The solution was then diluted to the mark with deionized water.

x) PORPH Stock Solution (1000 µM)

PORPH salt (0.618 mg) was weighed and transferred into a 1 litre volumetric flask. 600 cm^3 of deionized water was added with a magnetic stirrer till the salt completely dissolved. The solution was then diluted to the mark with deionized water.

xii) Chelating Ligands Standards

Standard solutions of 10 µM, 50 µM and 100 µM for each of the chelating ligands were made from their respective stock solutions and stored in sterilized amber-coloured bottles.

3.2 Methods

3.2.1 Ash Content Determination (AOAC, 1980)

The crucibles were weighed with an analytical digital balance (Sartorius, USA), kept in desiccators and the weight was recorded as W_1. Two grammes of the ground waste sample were each transferred into the crucible and the weight was taken as W_2. The sample with the crucible was then placed in a muffle furnace (Carbolite, UK) and the temperature was set to 550°C. The samples incinerated in the furnace for 8hours; the crucibles were then removed, cooled in desiccators and the weights recorded as W_3. The percentage of ash content was then calculated using the formula:

$$\% \text{ Ash} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times \frac{100}{1}$$

$$= \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

3.2.2 Moisture Content Determination (AOAC, 1980)

Crucibles were washed and dried to a constant weight in an oven (Gallenkamp, UK) at a temperature of 60°C. They were later removed, cooled in a desiccator, and re-weighed (W_1). Two grammes each of the ground samples were transferred into the weighed crucibles and the new weight taken as W_2. The crucibles containing the samples were then kept in the oven at a temperature of 100°C for 24hrs, and then weighed. The sample was returned into the oven to be re-weighed after about 3hrs and the weight recorded as W_3. The percentage moisture content was then calculated using the formula:

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

3.2.3 Determination of Lipids Content (AOAC, 1980)

Procedure: About 200 cm^3 of petroleum ether (Boiling point 40 – 60^oC) was transferred into a clean and dried 250 cm^3 capacity round bottomed flask containing some antibumping granules fitted with a Soxhlet extraction unit connected to a condenser. The extraction thimble was weighed using an analytical digital balance (Sartorious, USA) and the weight was recorded as (W_1). 20 g of the sample was then transferred into the thimble and weighed (W_2). The thimble was then fixed into the soxhlet extraction unit with forceps and the condenser connected. The heating mantle was switched on and the temperature adjusted between 40^oC to 60^oC; until the solvent refluxing was at a steady rate.

Extraction was then carried out for 8 hours after which the heating mantle was switched off. The thimble was removed and dried to a constant weight in an oven at 70^oC and re-weighed (W_3).

The percentage of extractible lipid was calculated using the formula:

$$\frac{Weight\ of\ lipid\ extracted}{Weight\ of\ Sample} \times 100\% = \frac{W_2 - W_3}{W_2 - W_1} \times 100.$$

3.2.4 Nitrogen Content Determination (Kjedahl method)

The nitrogen content determination was carried out in three stages thus:

Digestion: Two gram of the sample was weighed into a dried Kjeldahl Flask (500 cm^3), one gram of mixed (K_2SO_4 and $CuSO_4$) was added as catalyst, together with 6 cm^3 of concentrated H_2SO_4 and a few anti-bumping granules. The mixture was then heated until the greenish solution turned colourless. After cooling, the solution was transferred into a 100 cm^3 volumetric flask and diluted to mark with distilled water.

Distillation: 10 cm^3 of the above solution was transferred into a Kjeldahl distillation apparatus where 10 cm^3 of 40 % sodium hydroxide solution was added to liberate ammonia. The ammonia liberated was received in 100 cm^3 conical flask containing 5 cm^3 of 40 % boric acid and 2 drops of methyl red indicator. The distillation continued until the pink colour of the indicator turned greenish in colour.

Titration: The content of the conical flask was then titrated with 0.1 M HCl with end point indicated by a change from green to pink colour. The volume of the acid used for each sample titration was noted as well as that of the blank. The % nitrogen content was then calculated using the formula:

$$\text{Total Nitrogen (\%)} = \frac{(V_1 - V_0) \text{ X M X } 14 \text{ X } 100 \text{ X } 100}{W \text{ X } 1000 \text{ X } 10}$$

Where W = weight of sample

V_1 = Volume of HCl required to neutralize 10 cm^3 of sample solution

V_O = Volume of HCl required to neutralize 10 cm^3 of blank

14 = Atomic mass of nitrogen

100 = Total volume of digest

100 = Percentage conversion

M = Molarity of HCl (0.1 M)

1000 = Conversion to litre

10 = Volume of distillate

Note: Percentage of crude protein = % N × 6.25;

Where 6.25 is the conversion factor for protein (equivalent to 16 g nitrogen per gramme of protein).

3.2.5 Carbohydrate Content Determination (Pearson, 1976)

The percentage carbohydrate was calculated by simply subtracting the sum total of protein, moisture, ash and lipid content from 100 for each waste sample.

% Carbohydrate (CHO) = 100 – (% moisture + % ash + % crude protein +crude lipid).

3.2.6 Total Solids Determination (Hames *et al.*, 2003)

Crucibles were pre-dried in an oven (Gallenkamp, UK) at a temperature of 105°C for a period of about 4 hours. The dishes were then cooled in a desiccator then weighed using an analytical digital balance (Sartorius, USA) and the weight recorded as W_1. Two grammes of each samples was weighed into the crucibles. The weight of the sample plus the weighing dish was taken using the analytical balance and recorded as W_2. The samples were then placed in the oven at a temperature of 105°C for a minimum of four

hours. Afterwards, the samples were removed and allowed to cool at room temperature in a desiccator. The weighing dishes containing the oven-dried samples were weighed, returned to the oven to be re-weighed after about 1hour and the final weight was taken as W_3. The % total solid (TS) was calculated using the formula:

$$\% \text{ TS} = \frac{(\text{Weight}_{\text{dry crucible + dry samples}}) - (\text{Weight}_{\text{dry crucible}}) \text{ X } 100}{\text{Weight}_{\text{sample}}}$$

3.2.7 Volatile Solid Determination (Hames *et al*., 2003)

The dried residue from total solids analysis was weighed and heated in a crucible for two hours at 500^{o}C in a preheated Muffle furnace (Carbolite, UK). After cooling, the crucible and ash were weighed.

Volatile solids (VS) % = $100 - ((V_3-V_1)100 / (V_2-V_1))$

Where V_1 = weight of the crucible

V_2 = weight of dry residue and crucible

V_3 = weight of ash and crucible (after cooling)

3.2.8 Determination of Trace Metals in Substrates

One gramme of each of the various substrates was placed in a 250 cm^3 pyrex beaker and 10 cm^3 of concentrated nitric acid was added. The samples were then heated for forty-five minutes at a temperature of 90 °C; the temperature was then increased to 100 °C at which the samples were allowed to boil for about eight hours until a clear solution was obtained. 15 cm^3 of concentrated nitric acid was added to each mixture where digestion occurred until the volume was reduced to about 1 cm^3. The interior wall of the beakers was washed down with a little deionized water and the beakers swirled throughout the digestion to keep the wall clean and prevent loss of

sample. After cooling, 5 cm^3 of 1% nitric acid was added to the sample. The solutions were each filtered with no 42 filter paper < 0.45 millipore and the filterates transferred quantitatively into a 25 cm^3 volumetric flask where they were each made up to the mark with deionized water. The concentration of Nickel (Ni), Cobalt (Co), Iron (Fe), Manganese (Mn) and Zinc (Zn) in the final solutions were determined spectrometrically by the use of the Atomic Absorption Spectrophotometer (AAS).

3.2.9 Method for Monitoring System Performance

The performance of the various digester systems was assessed by monitoring the CH_4- production, pH, VFA- concentration and VS- reduction as recommended by Pind *et al.,* 2003. The five digesters labelled GU. BL, CH, CM and CD were operated over a period of 50 days at a mesophilic temperature range of 29 - 31°C. The weights of the five different substrates were varied from 150 to 250 g and the percentage CH_4 gas yield at the optimized weights was obtained for each substrate.

3.2.10 Method for Production and Measurement of Biogas

Two litres Pyrex digester bottles were used. Two hundred grammes of each dry sample were loaded into the digesters and 1.5 litres of deionized water was added. This was sealed with a rubber bung having two bore holes. One hole was used for temperature determination, while the other was connected to delivery tubing which was used to collect and measure the volume of biogas produced under water through the downward displacement of water method. The digesters were subjected to periodic agitation to ensure thorough mixing of the contents while maintaining intimate contact between

the micro-organisms and the substrate to enhance the complete digestion of the substrate. The composition of the biogas produced was equally monitored using a biogas analyzer (Biogas 5000, UK) on a daily basis.

The above procedure was repeated for the determination of the effect of chemical additive in the biogas yield; however, in this case, the substrates were introduced into the digesters; each containing solutions of the chelating ligands. Plate 3.1 shows the setup for the biogas production.



Plate 3.1: The Experimental Setup for the Biogas Production.



Plate 3.2: Picture of the Biogas 5000 Analyser

3.2.11 VFA and pH Determination (Kapp, 1984)

The VFA analysis was carried out to determine the concentration of VFA (e.g. acetic acid, propionic acid, butyric acid e.tc.) present within each of the five digester systems. The pH analysis was performed in each of the GU, BL, CM CD and CH digesters to ascertain the H^+ ion concentrations within each digester system.

Before analysis, the samples from each digester were filtered through a 0.45 µM membrane filter. 20 cm^3 of the filtrates were transferred into a 100 cm^3 Pyrex beaker and the initial pH values of the filtrates determined using the pH meter (Jenway 3505, UK). The samples were titrated slowly with 0.05M sulphuric acid until pH of 5.0 was reached. The added volume of titrant was recorded. More acid was slowly added until pH of 4.3 was reached. The total volume of the added titrant was again recorded. The latter step was repeated until pH of 4.0 was reached; the volume of the added titrant was again recorded. The concentration of VFA was then calculated using the formula:

$$Sa = \frac{131340 \,.\, M \,.\, VA_{5-4,\,meas}}{Vs} - 3.08 \,.\, Alk_{meas} - 25$$

Where Sa = concentration of VFA; M = Molarity

$VA_{5-4,\,meas}$ = volume of acid required to titrate a sample from pH 5.0 - 4.0

Vs = volume of sample

Alk_{meas} = measured alkalinity

$$[Alk_{meas} = \frac{VA_{4.3\,meas} \,.\, M. \,1000}{Vs}]$$

CHAPTER FOUR

4.0 RESULTS

4.1 Results of the VFA, pH and CH_4 gas Yield Analysis at Optimized Substrate Weights.

Figure 4.1 shows a plot of the CH_4 gas yield from the GU, BL, CD, CH, and CM digester systems against their optimized weights. The digester system labelled GU, recorded a maximum CH_4 gas yield of 8.30 % at a substrate weight of 150g. For the BL digester system, it showed a performance of 33.30% at an optimum weight of 150 g; CD digester - 67.40% at an optimum substrate weight of 150 g and the CH – 84.80% at an optimum substrate weight of 150 g. The only exception observed was with the CM digester system which recorded a maximum CH_4 gas yield of 75.40% with an optimum substrate weight of 250 g. Figure 4.2 shows a plot of the VFA concentration in mg/L within the GU, BL, CD, CH and CM digesters against time in weeks. The data given in Table 4.1 shows the pH values of the substrates taken on a weekly basis.

4.2 Proximate Analysis and Trace Metals Determination of Substrates

The concentration of trace metals present within the substrates GU, BL, CD, CH and CM was determined and the result presented in Figure 4.3. Proximate analysis of the substrates GU, BL, CD, CH and CM was performed to determine quantitatively the percentage of the macronutrients present within each substrate; this includes: the ash content, moisture content, total solid (TS), volatile solid (VS), carbohydrate and nitrogen content. The result of this analysis is presented in Table 4.2.

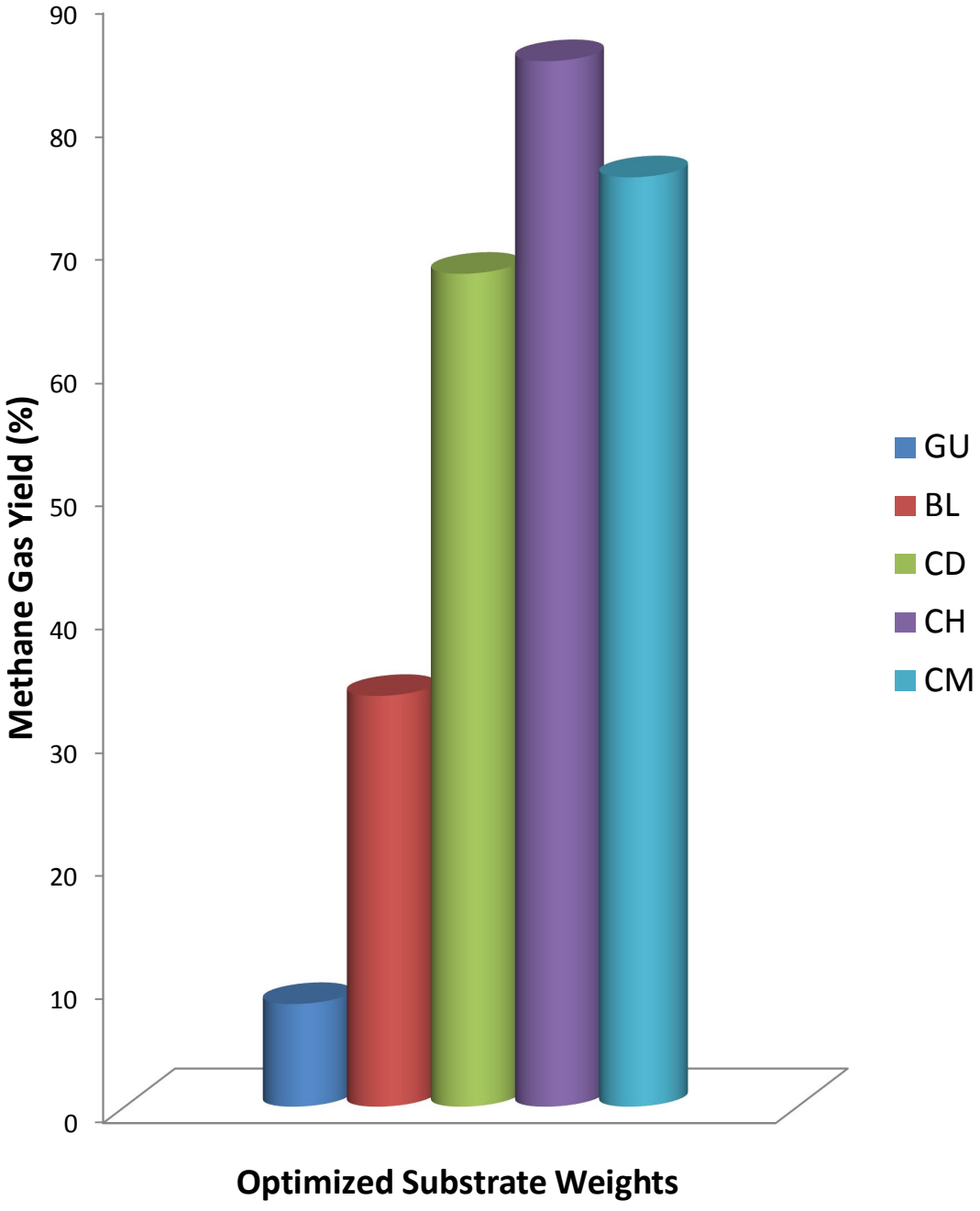


Figure 4.1: CH_4 gas Yield at Optimized Substrates Weight

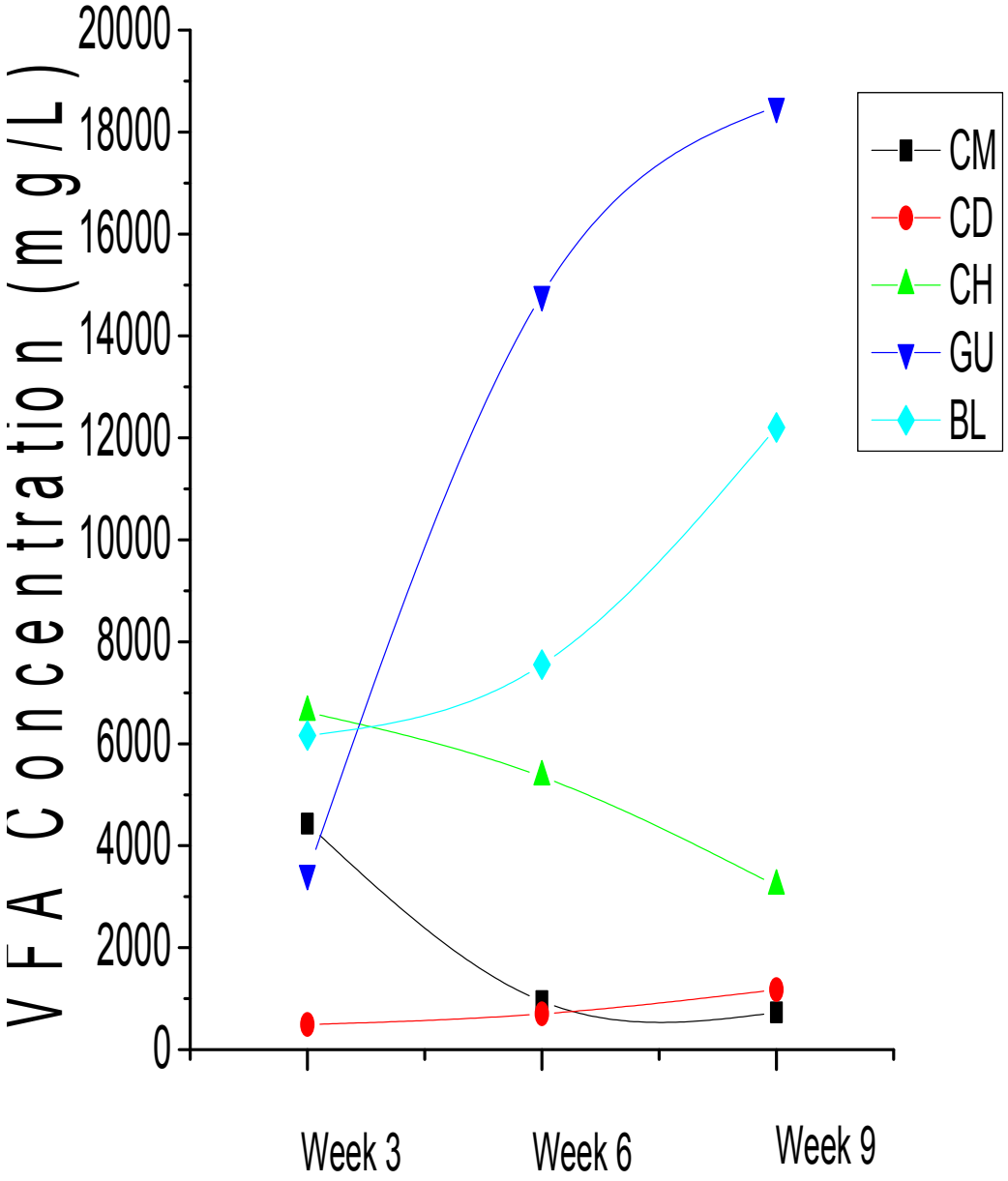


Figure 4.2: **VFA Concentration of Digester System, GU, BL, CM, CH and CD at Optimized Weights.**

Table 4.1: pH Values of the Substrates GU, BL, CM, CD and CH

WEEK	RESULTS				
	CM	**CD**	**CH**	**GU**	**BL**
3	6.54	6.95	6.65	4.35	4.96
6	6.90	6.90	7.03	5.21	5.14
9	6.77	6.60	7.30	5.16	5.15

Table 4.2: Physiochemical Properties of Substrates

PARAMETERS	RESULTS				
	GU	**CH**	**BL**	**CM**	**CD**
% Ash Content	10.51	41.68	32.59	53.28	13.52
% Moisture Content	4.89	5.00	7.23	4.03	6.18
% Total Solid	95.11	95.00	92.77	95.97	93.82
% Volatile Solid	19.10	42.53	48.61	50.34	53.82
%CHO	4.75	12.02	11.06	20.32	2.33
%N	0.30	2.00	0.69	2.54	0.21
C/N Ratio	16.00:1.00	6.00:1.00	16.00:1.00	8.00:1.00	11.00:1.00

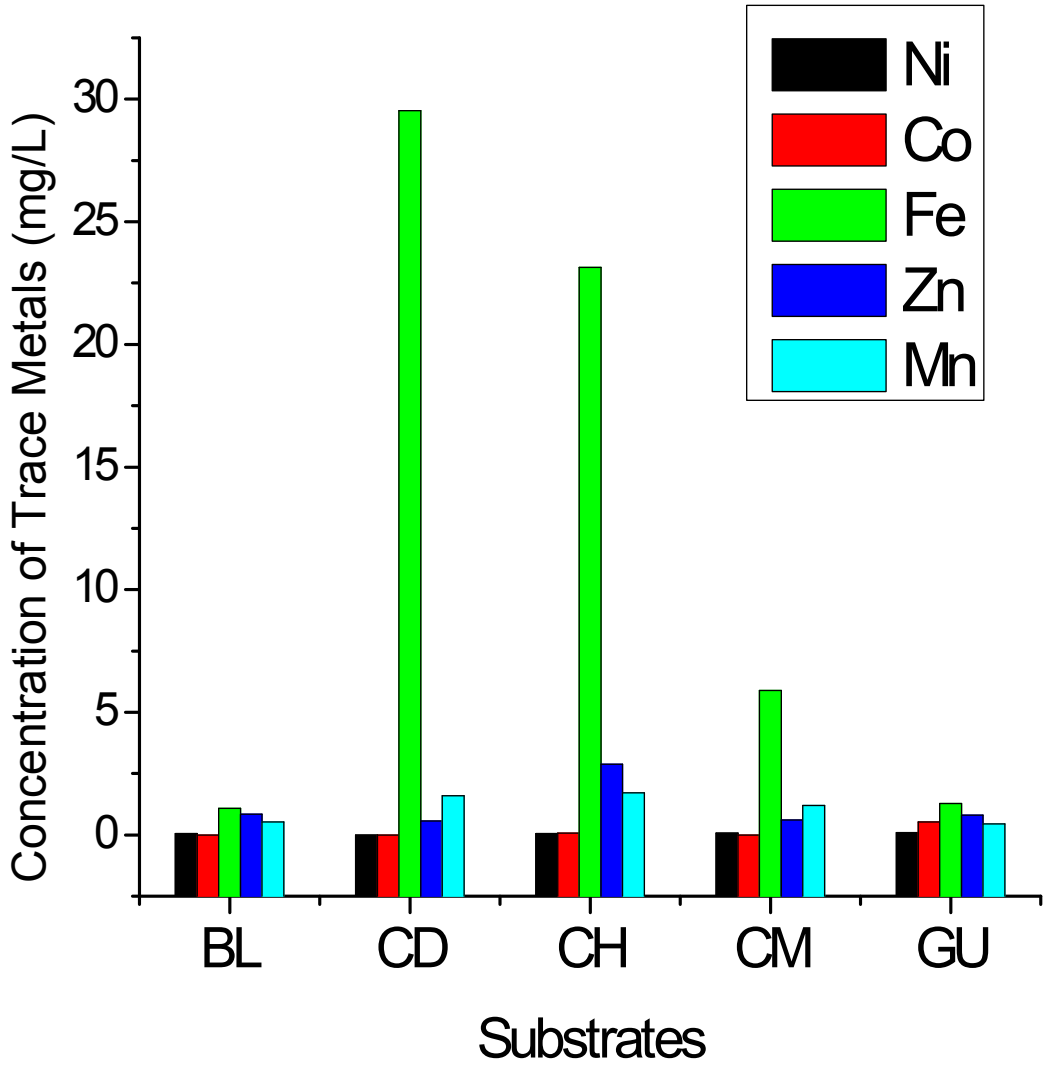


Figure 4.3: Concentration of Trace Metals within the Various Substrates

4.3 HRT of Optimized Substrates

The HRT which is the time interval when the substrate is kept in the digester was also determined for each of the five digester systems GU, BL, CM, CD and CH, and results presented in Figures 4.4 – 4.8.

4.4 Optimization of Chelating Ligand Ability, VFA Concentrations and pH Values

In a bid to improve on the performance of the three best performing digester systems CM, CD, and CH (i.e. digester systems where the maximum CH_4 gas yield was greater than 50%), the following chelating ligands were added to the substrates prior to the commencement of the anaerobic digestion process: EDDA, NTA, EDTA, DTPA, PORPH, MESYL and PENI. Figure 4.9 – 4.11 showed the plot of percentage methane gas yield against the seven chelating ligands labelled A - H in the CM, CD, and CH digester systems. The peak points in each of the digester systems indicated the highest CH_4 gas yield for each ligand present within each digester system. The VFA concentrations for the CM, CD, and CH digester systems where determined and presented in Figures 4.12 – 4.14. Figures 4.12 – 4.14 were obtained by plotting the various peak VFA concentrations value determine in mg/L within the CM, CD and CH digester systems against the seven chelating ligands labelled as A – H. The data of the various pH values obtained from the CM, CD and CH digester systems over a period of weeks are presented in Tables 4.3 - 4.5.

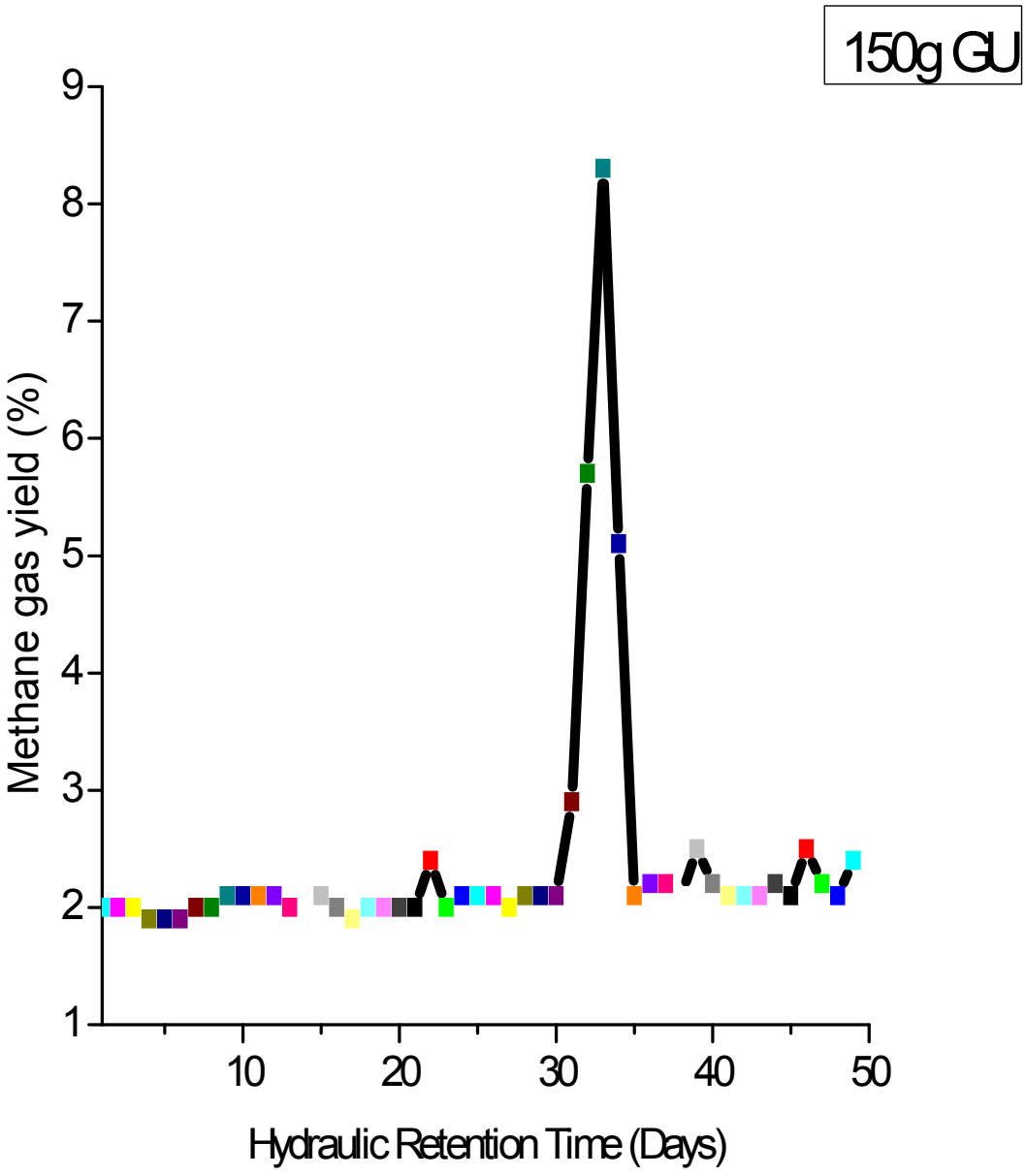


Figure 4.4: Methane Yield against Hydraulic Retention Time for the GU Substrate

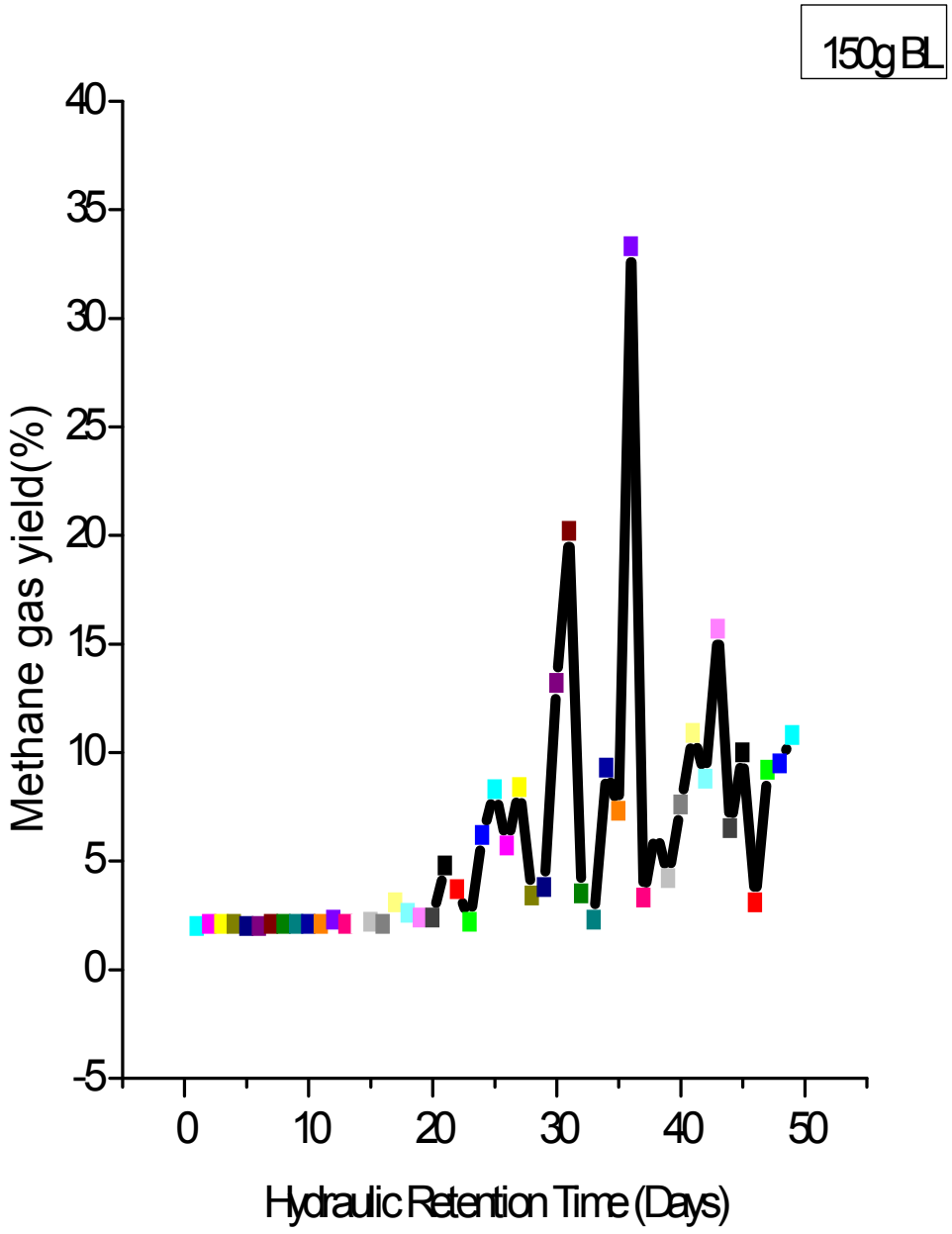


Figure 4.5: Methane Yield against Hydraulic Retention Time for the BL Substrate

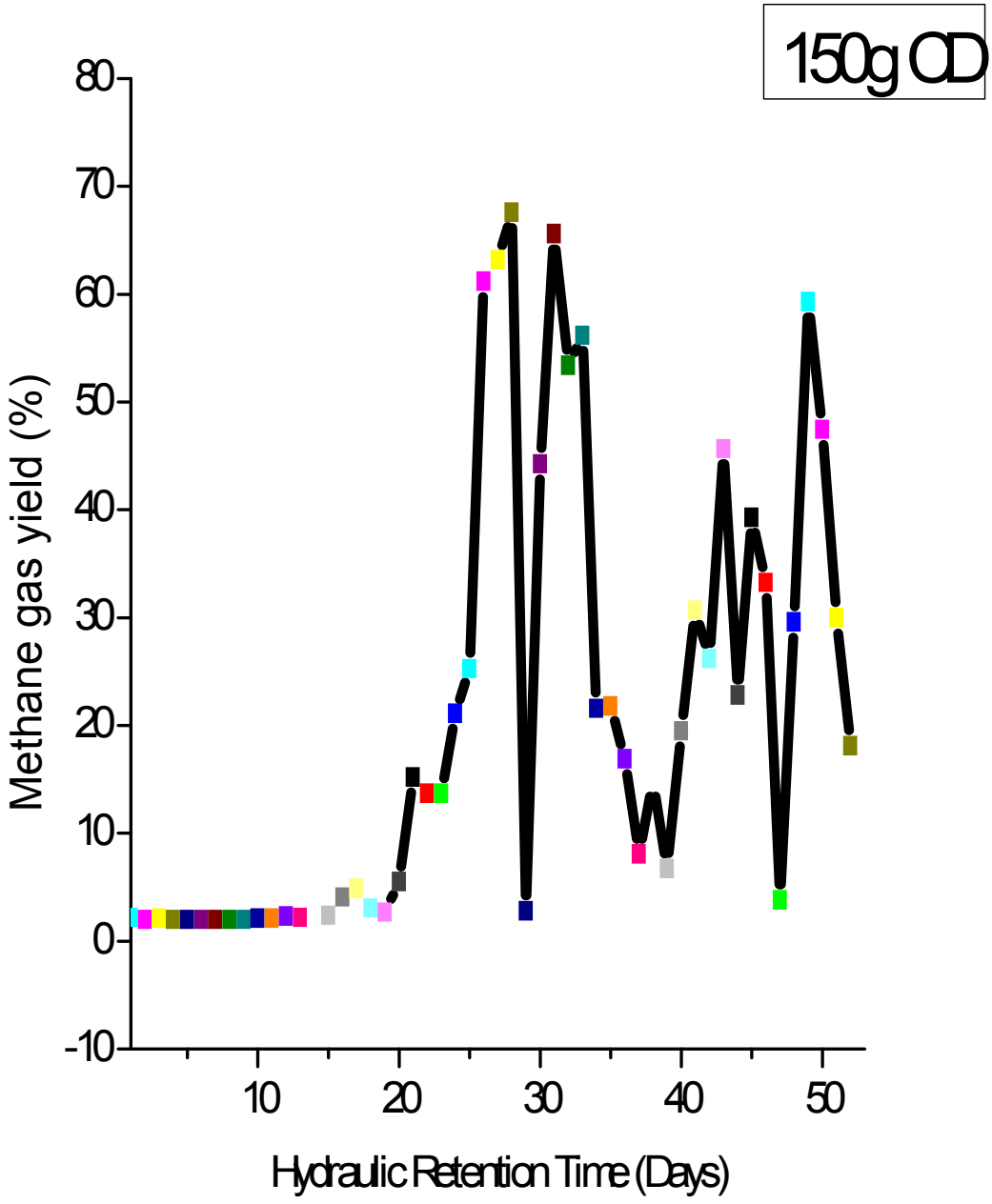


Figure 4.6: Methane Yield against Hydraulic Retention Time for the CD Substrate

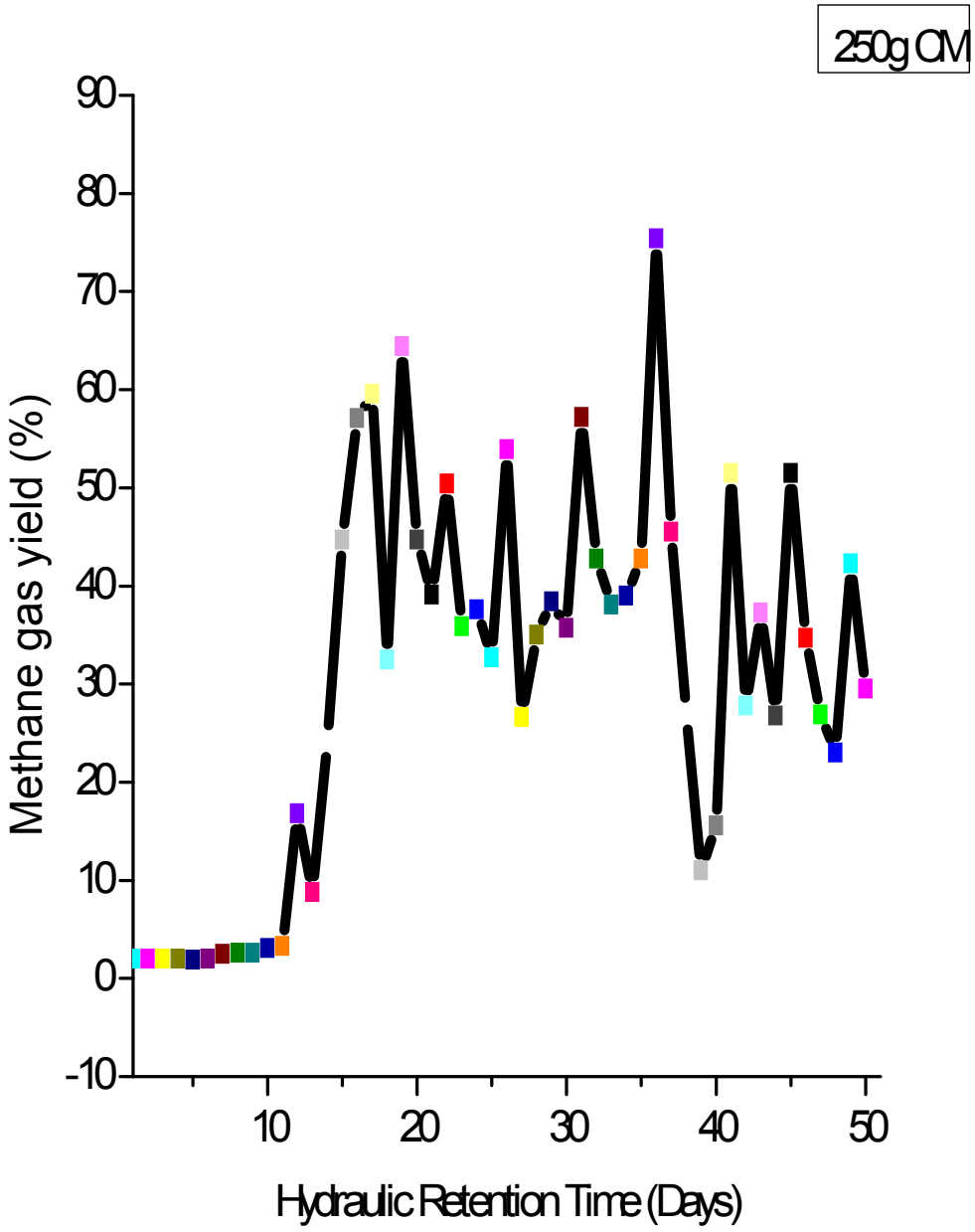


Figure 4.7: Methane Yield against Hydraulic Retention Time for the CM Substrate

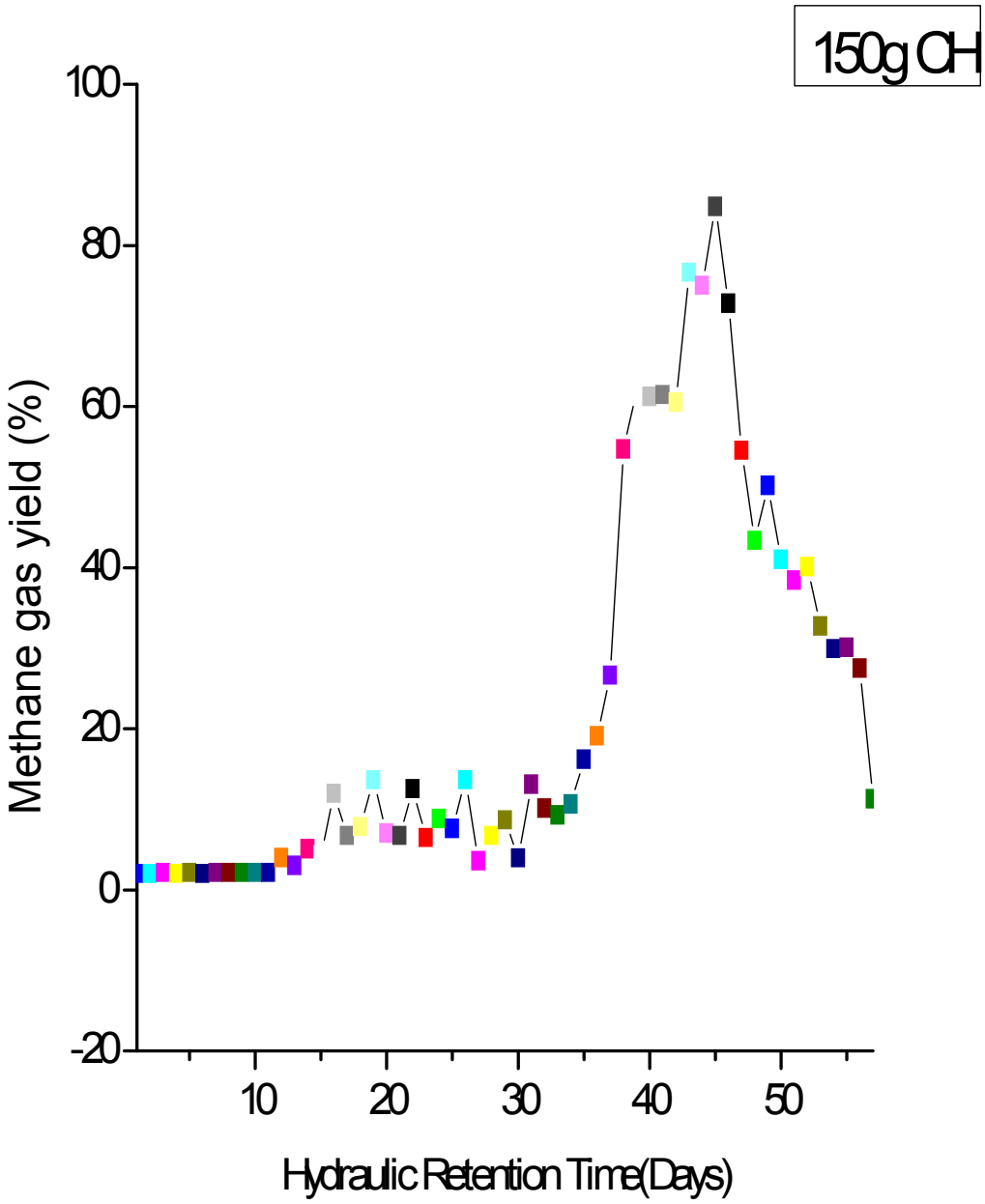
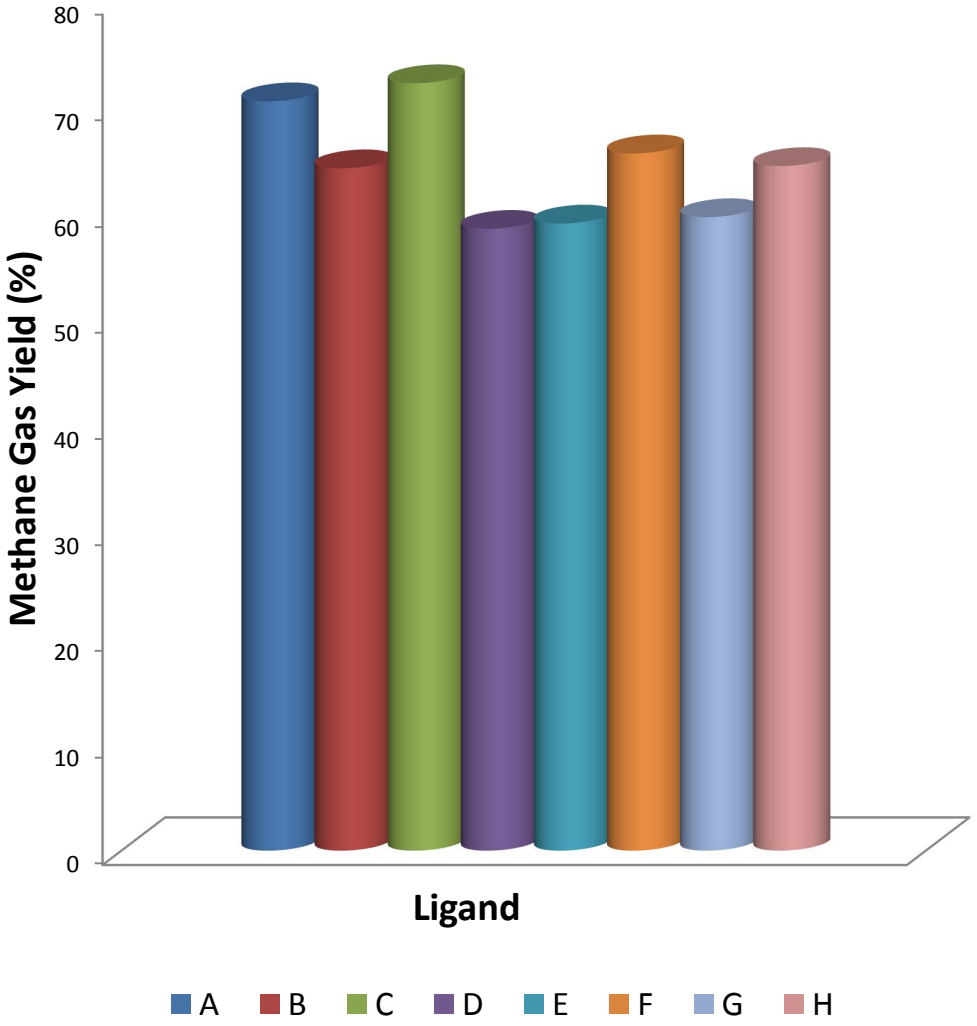
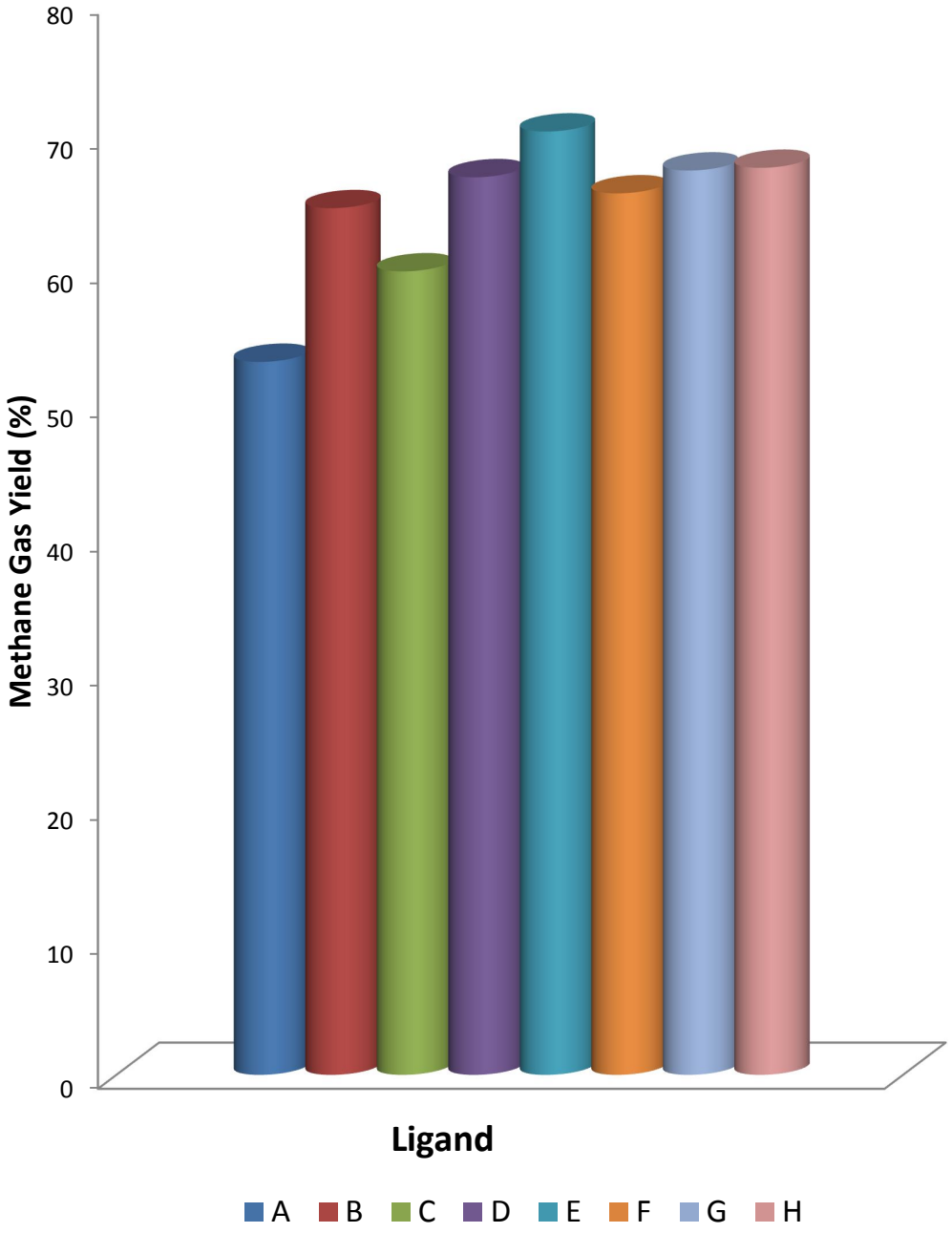


Figure 4.8: Methane Yield against Hydraulic Retention Time for the CH Substrate



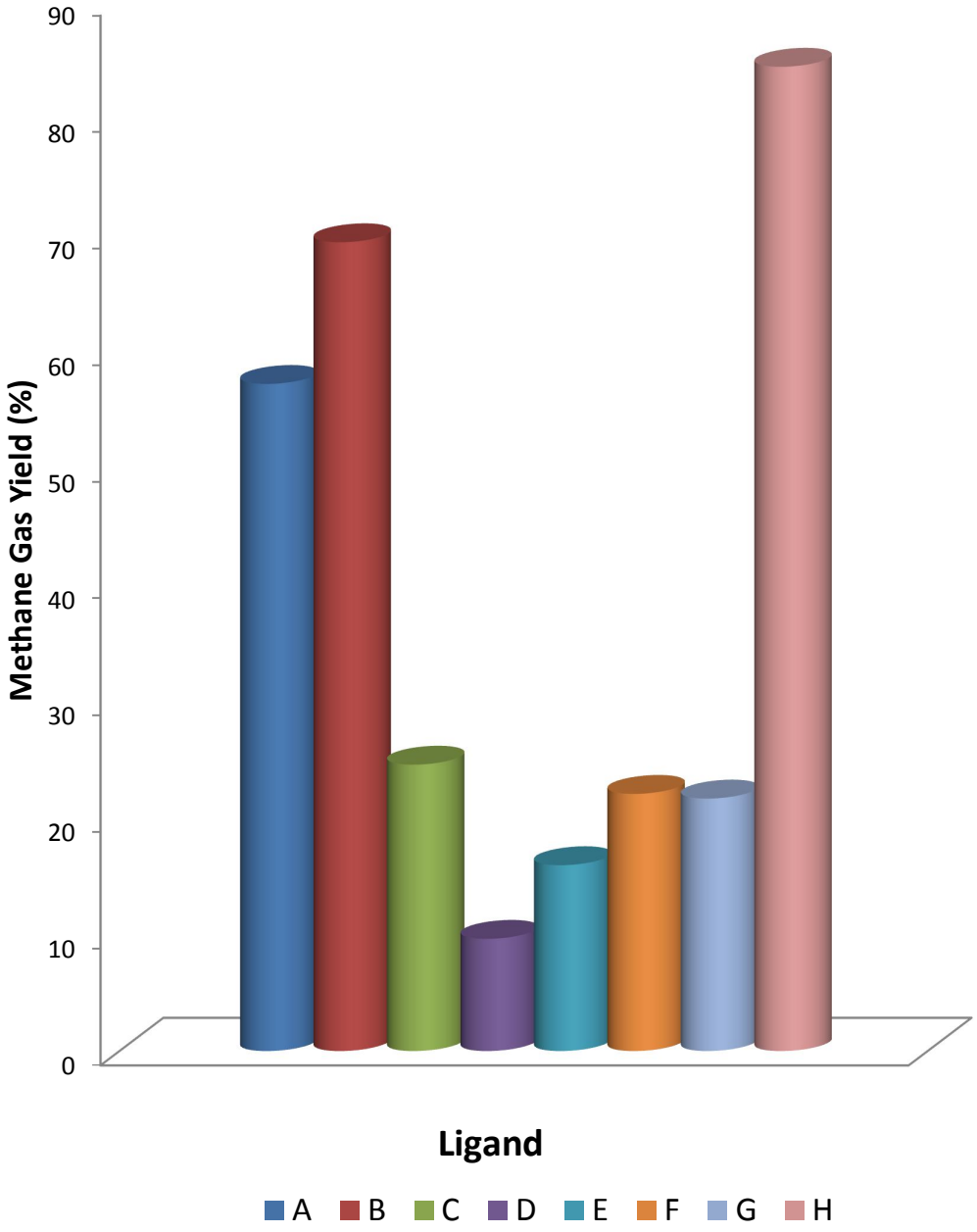
A=EDDA, **B**=MESYL, **C**=NTA, **D**=PENI, **E**=EDTA, **F**=DTPA, **G**=PORPH, **H**=Control

Figure 4.9: Optimization of the Chelating Ligand Ability within the CM digester



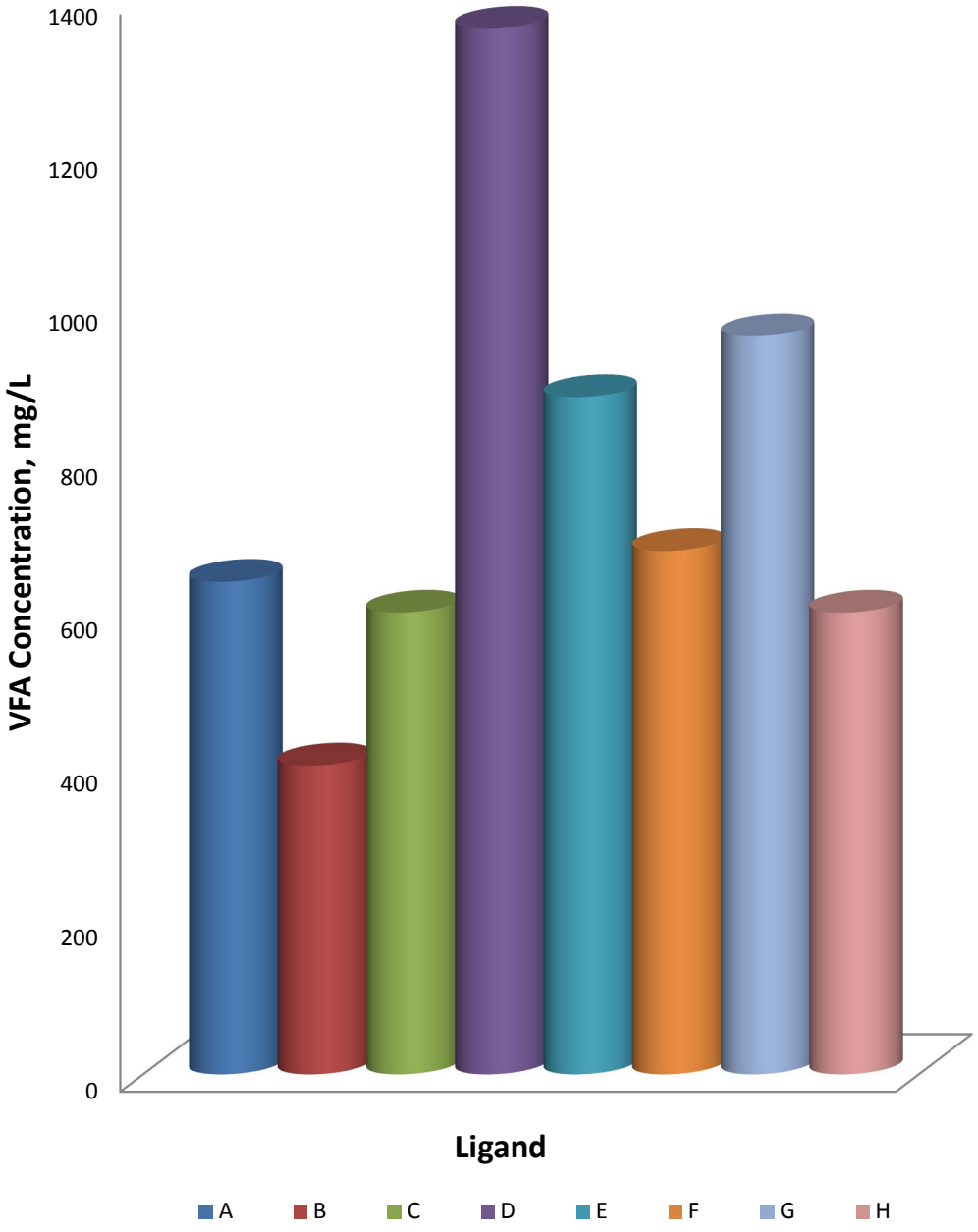
A=EDDA, **B**=MESYL, **C**=NTA, **D**= DTPA, **E**=EDTA, **F**=PENI, **G**=PORPH, **H**=Control

Figure 4.10: Optimization of the Chelating Ligand Ability within the CD digester



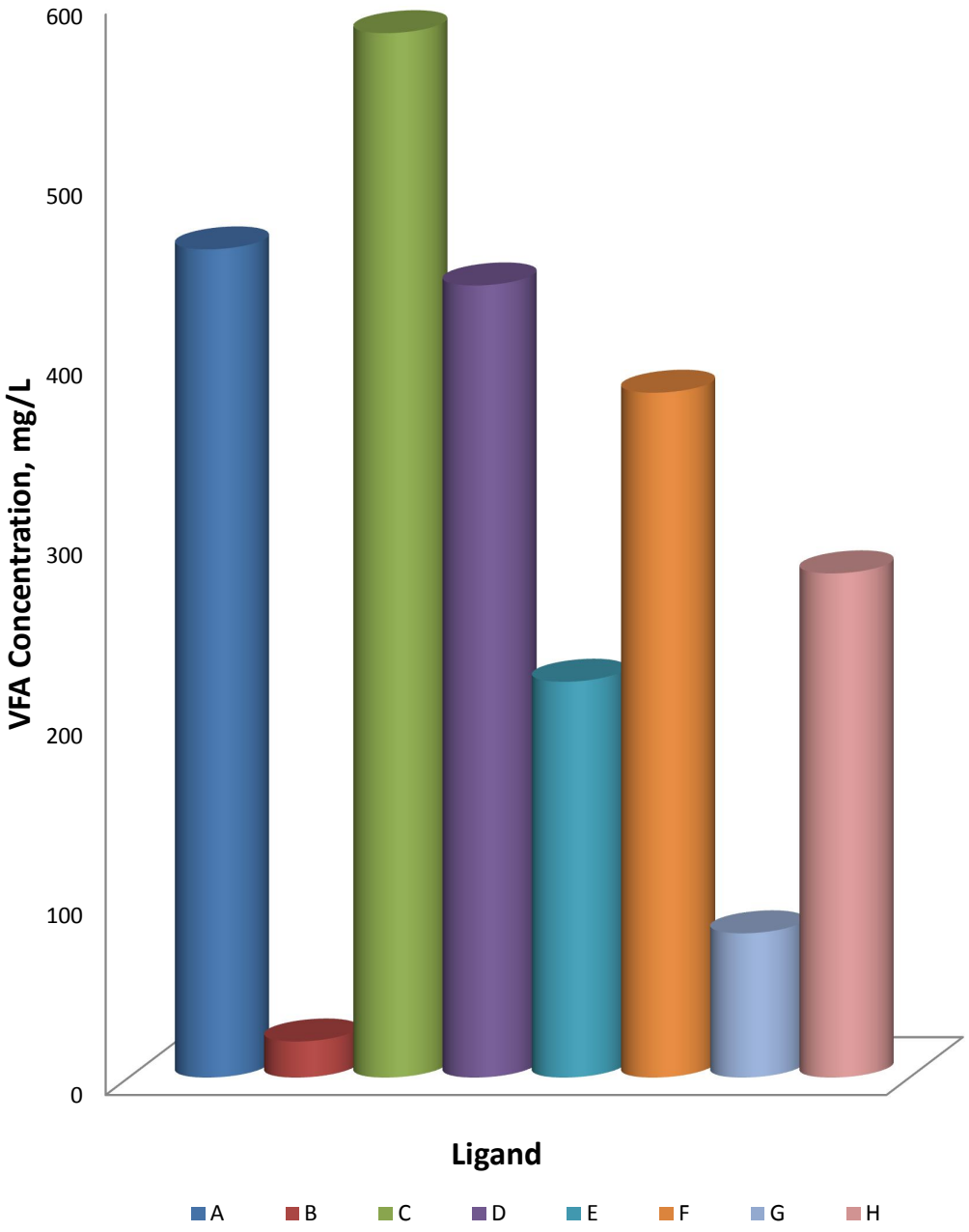
A= EDDA, **B**=MESYL, **C**=DTPA, **D**=PENI, **E**=EDTA, **F**=NTA, **G**=PORPH, **H**=Control

Figure 4.11: Optimization of the Chelating Ligand Ability within the CH digester



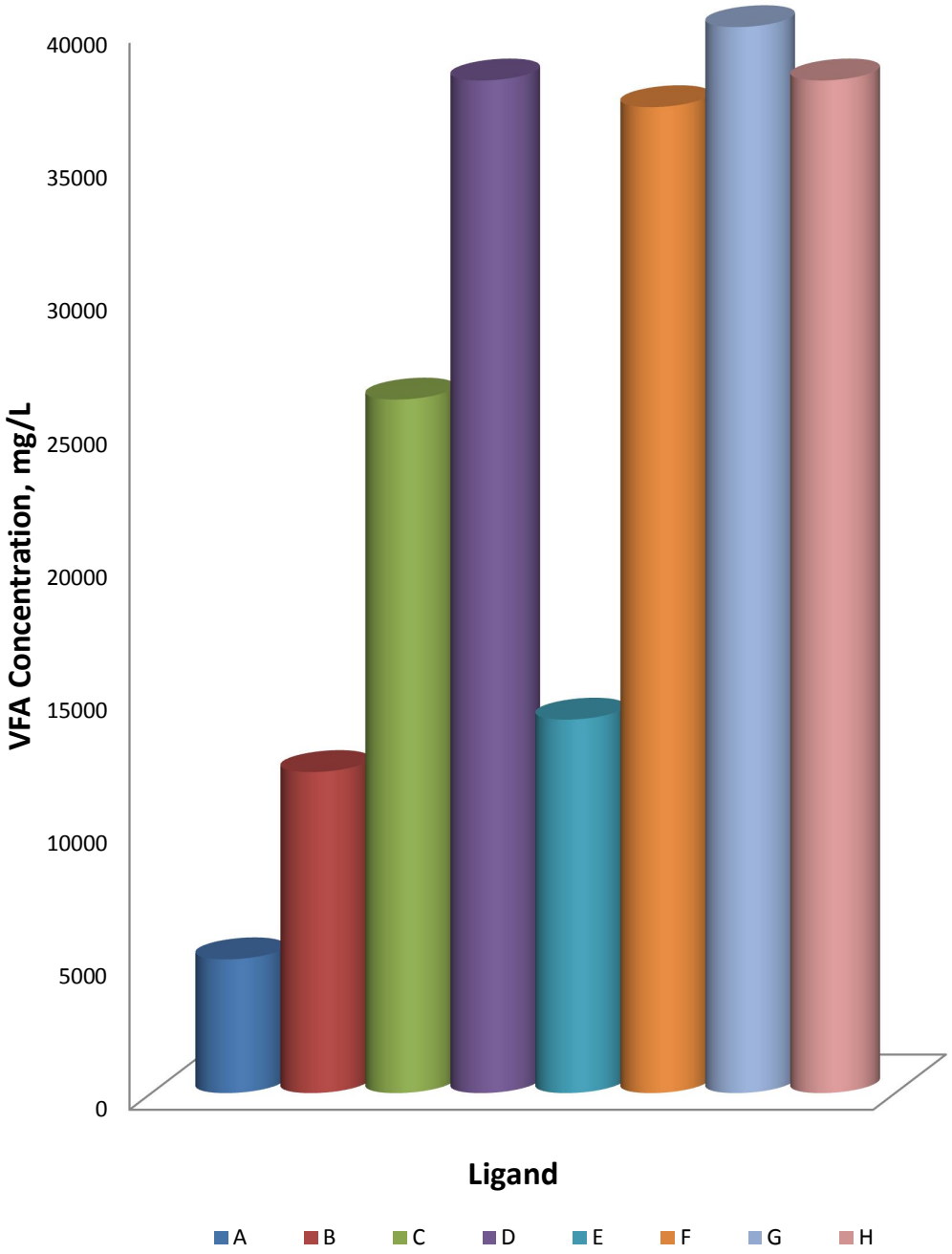
A= Control, **B**= EDDA, **C**=NTA, **D**=EDTA, **E**=MESYL, **F**=DTPA, **G**=PORPH, **H**=PENI

Figure 4.12: Effect of Addition of Chelating Ligand on VFA Concentration in CM Digester



A= Control, **B**=EDDA, **C**=NTA, **D**=EDTA, **E**=MESYL, **F**=PENI, **G**=PORPH, **H**=DTPA

Figure 4.13: Effect of Addition of Chelating Ligand on VFA Concentration in CD Digester



A=Control, **B**= EDDA, **C**=DTPA, **D**=EDTA, **E**=MESYL, **F**=NTA, **G**=PORPH, **H**=PENI

Figure 4.14: Effect of Addition of Chelating Ligand on VFA Concentration in CH Digester

Table 4.3: Effect of Addition of Chelating Ligand on pH for CM Substrate

WEEK	EDDA	NTA	EDTA	MESYL	DTPA	PORPH	PENI
1	6.04	6.00	6.05	5.92	6.15	6.09	6.00
2	7.37	7.38	7.54	7.37	7.95	7.41	7.66
3	7.50	7.37	7.49	7.33	7.43	7.45	7.35

Table 4.4: Effect of Addition of Chelating Ligand on pH for CD Substrate

WEEK	EDDA	NTA	EDTA	MESYL	DTPA	PORPH	PENI
1	6.81	7.33	7.42	7.28	7.41	6.93	7.36
2	7.26	7.29	7.29	7.25	7.44	7.16	7.34
3	7.12	7.43	7.25	7.12	7.35	7.19	7.31

Table 4.5: Effect of Addition of Chelating Ligand on pH for CH Substrate

WEEK	EDDA	NTA	EDTA	MESYL	DTPA	PORPH	PENI
1	5.90	5.83	6.12	5.76	6.26	6.45	6.36
2	6.16	5.90	5.87	6.18	6.09	6.00	5.94
3	7.39	5.88	5.78	7.24	6.01	6.19	5.83

4.5 Effect of Chelating Ligand on HRT

The seven chelating ligands EDDA, NTA, EDTA, DTPA, PORPH, MESYL and PENI, where again introduced into the digester systems of the best three performing substrates i.e. CM, CD, and CH. And their effects on the HRT within these digester systems monitored. Figure 4.15a - 4.17c are plots of percentage CH_4 gas yield from the different substrates, each containing the seven chelating ligands against time expressed in days.

4.6 Effect of Increasing Chelating Ligand Concentration on CH_4 gas Yield

A study on the effect of increasing concentration of chelating ligands was equally carried out on the best performing chelating ligand systems. From all the seven chelating ligands introduced into the CM, CD and CH digesters, only EDDA, NTA, and DTPA performed better compared to the other ligands; for the CM digesters; EDTA, DTPA and PORPH for the CD digesters and lastly, EDDA, DTPA and MESYL performed better compared to the other ligands for the CH digesters. Figures 4.18 – 4.20 shows the plot of percentage CH_4 gas Yield generated from each digester system against increasing chelating ligand concentration, from a concentration of 10 μM to 100 μM.

4.7 Effect of Addition of Chelating Ligand on H_2S Concentration

The concentration of H_2S gas generated within the CM, CD and CH digesters on increasing Chelating ligand concentration, was also studied and results recorded in Figures 4.21 -.4.23. The Figures 4.21 – 4.23 were obtained by plotting a graph of H_2S gas concentration produced from digester systems containing chelating ligand against increasing chelating ligand concentration, from a concentration of 10 μM to 100 μM.

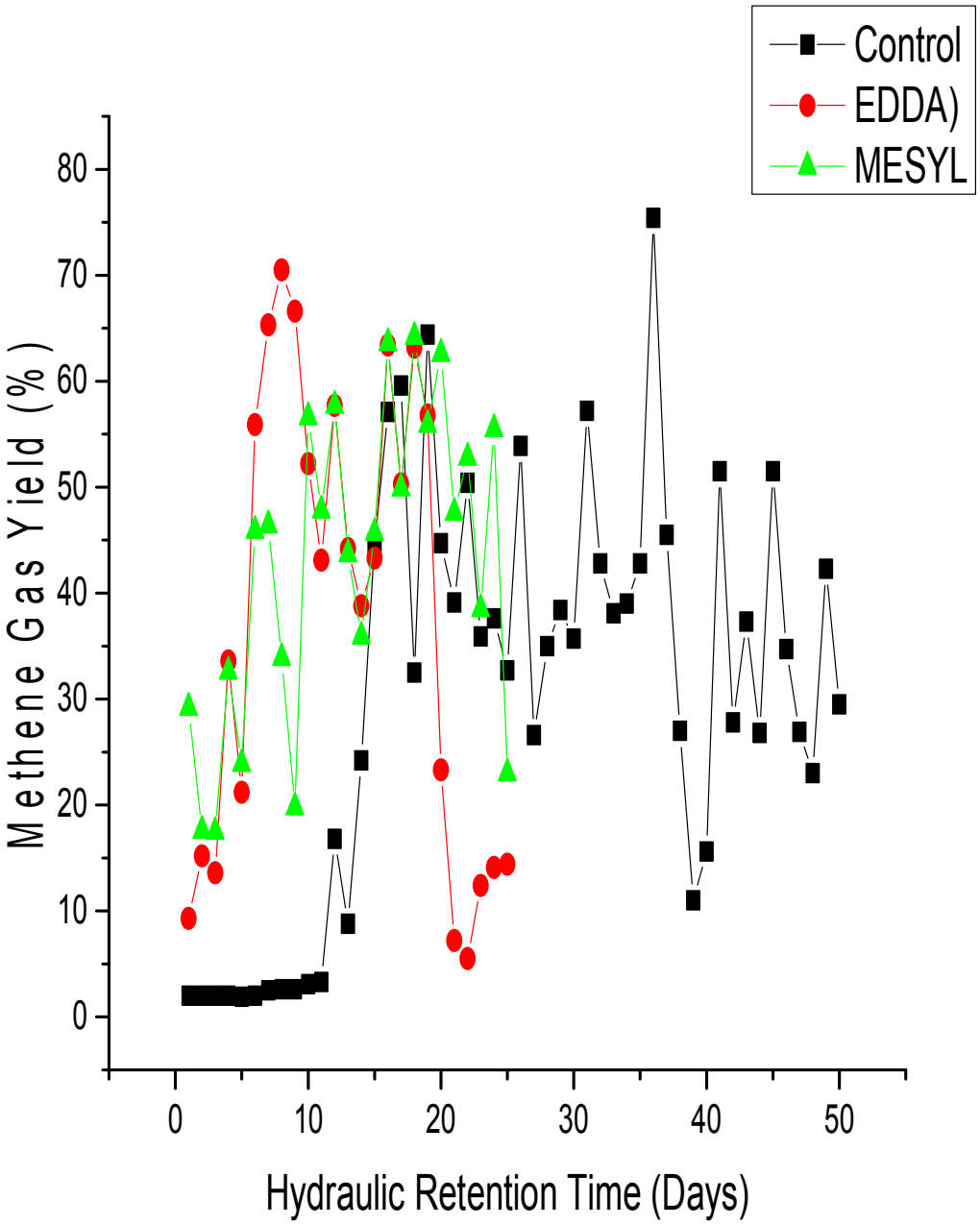


Figure 4.15a: Effect of Addition of Chelating Ligand on HRT in the CM-EDDA and MESYL Digester System

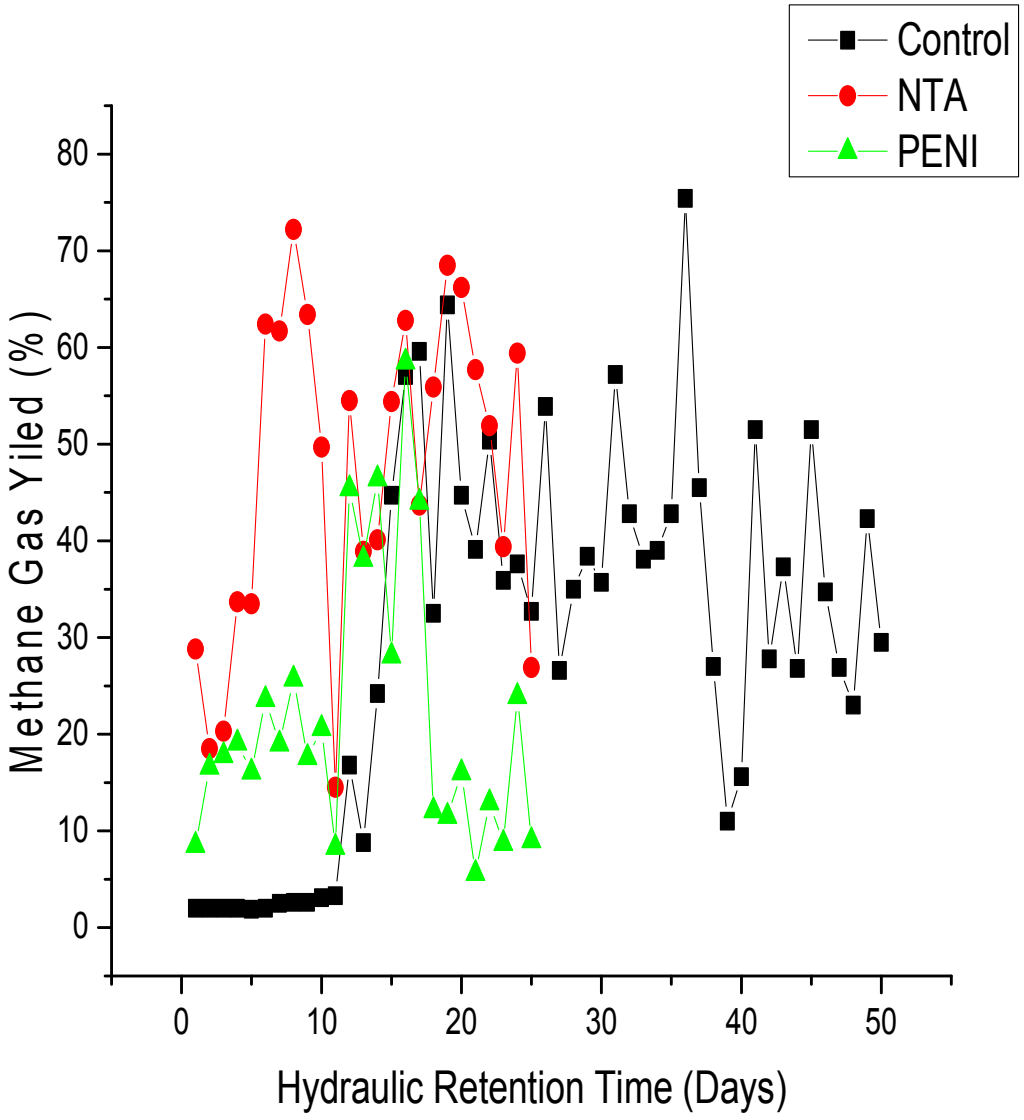


Figure 4.15b: Effect of Addition of Chelating Ligand on HRT in the CM-NTA and PENI Digester System

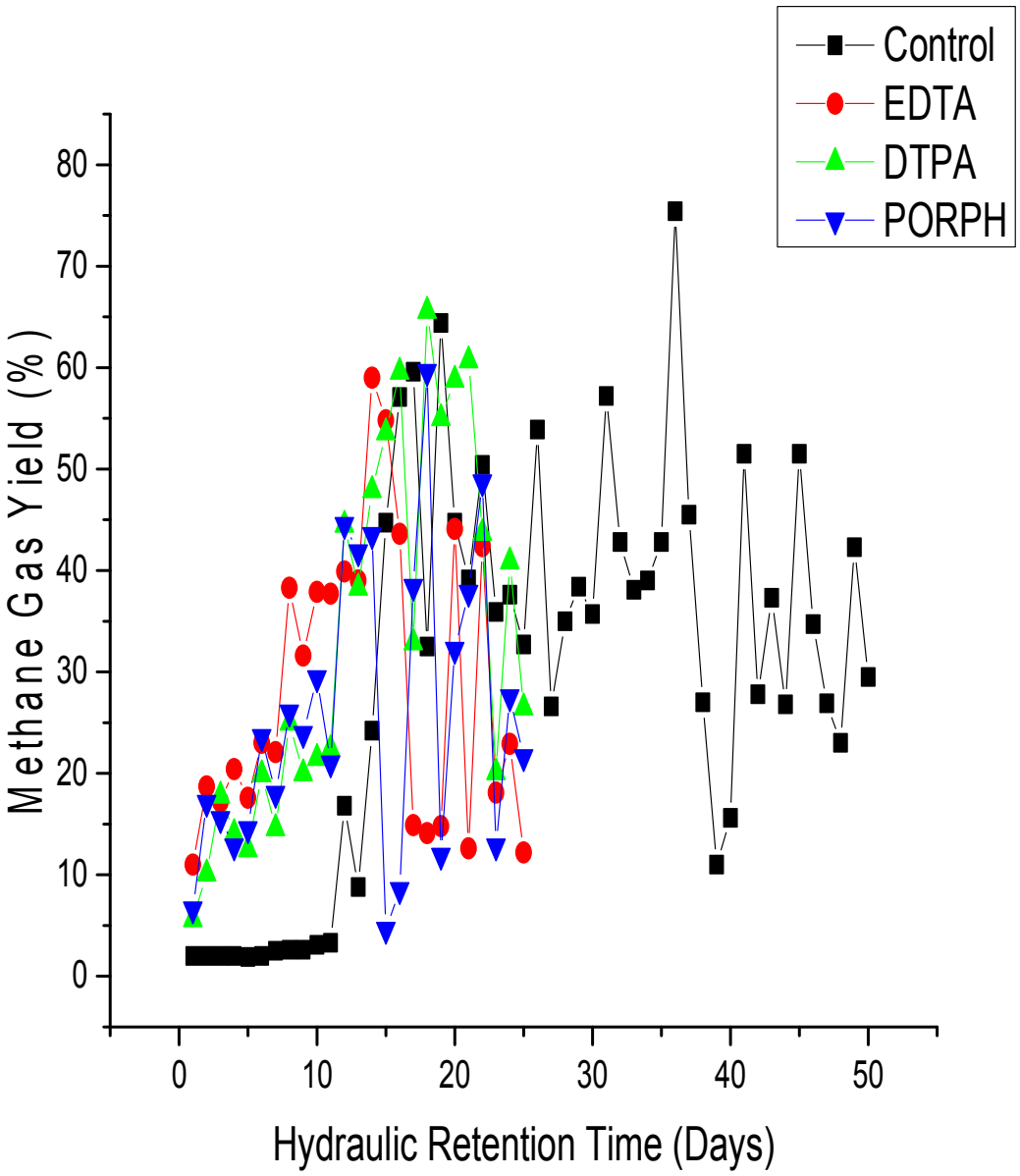


Figure 4.15c: Effect of Addition of Chelating Ligand on HRT in the CM-EDTA, DTPA and PORPH Digester System

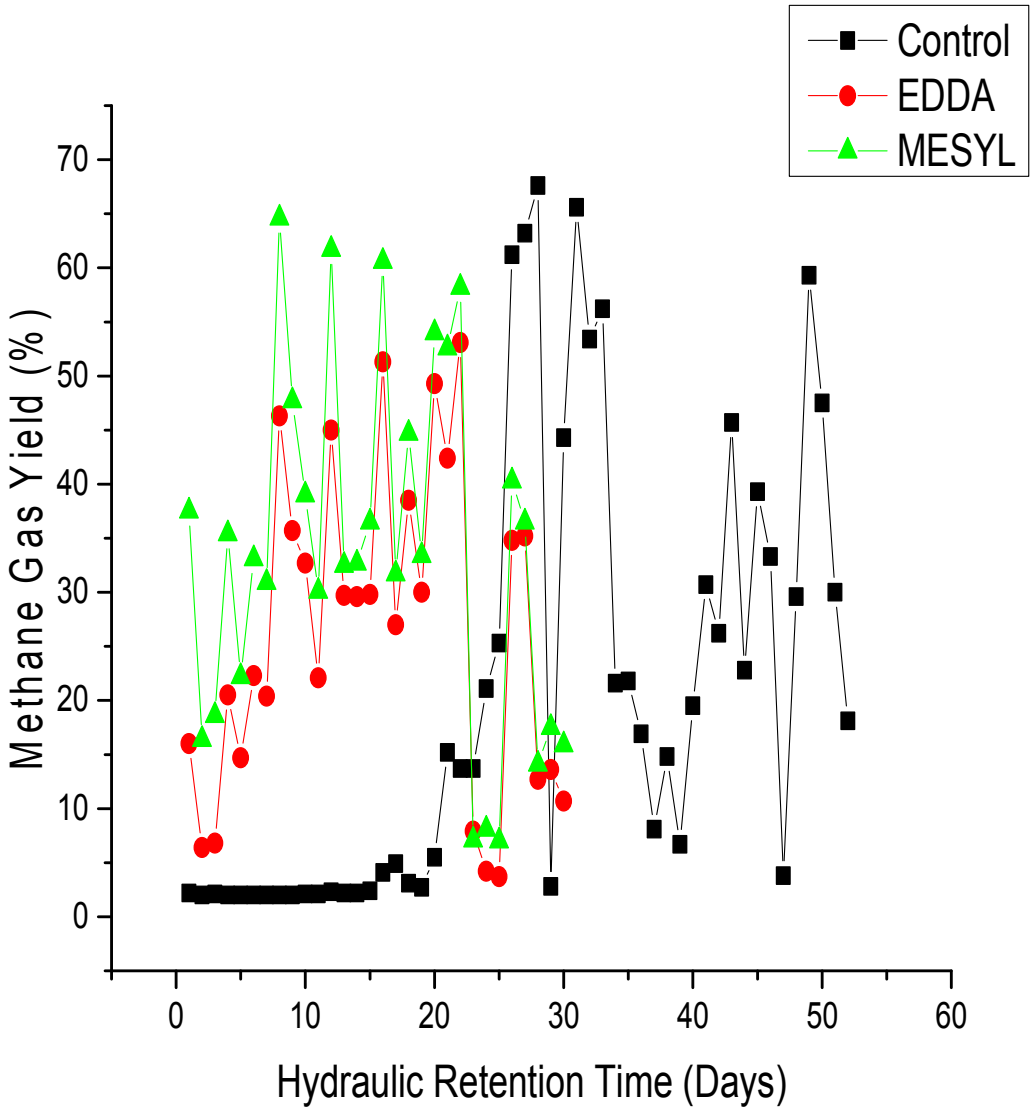


Figure 4.16a: Effect of Addition of Chelating Ligand on HRT in the CD-EDDA and MESYL Digester System

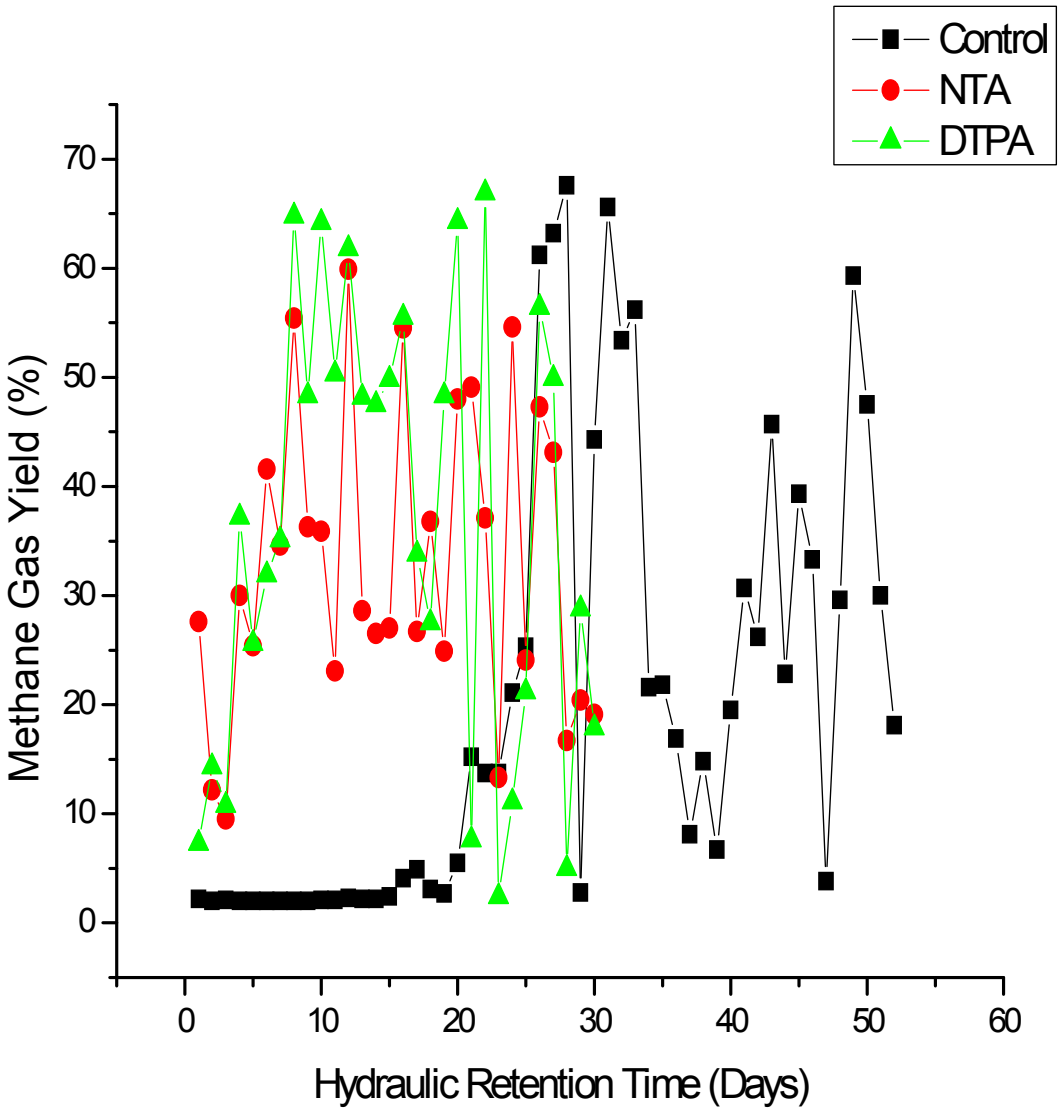


Figure 4.16b: Effect of Addition of Chelating Ligand on HRT in the CD-NTA and DTPA Digester System

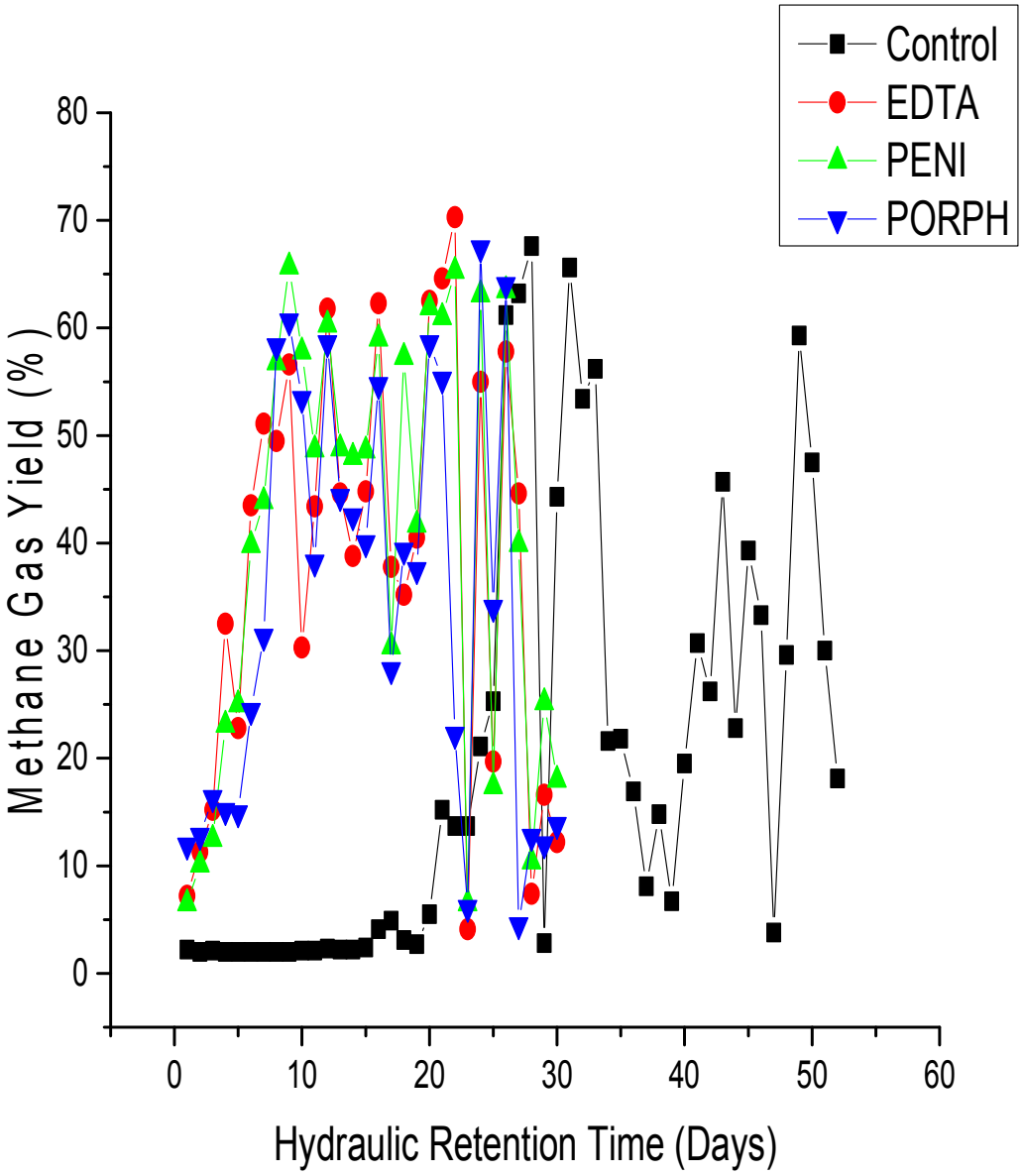


Figure 4.16c: Effect of Addition of Chelating Ligand on HRT in the CD-EDTA, PENI and PORPH Digester System

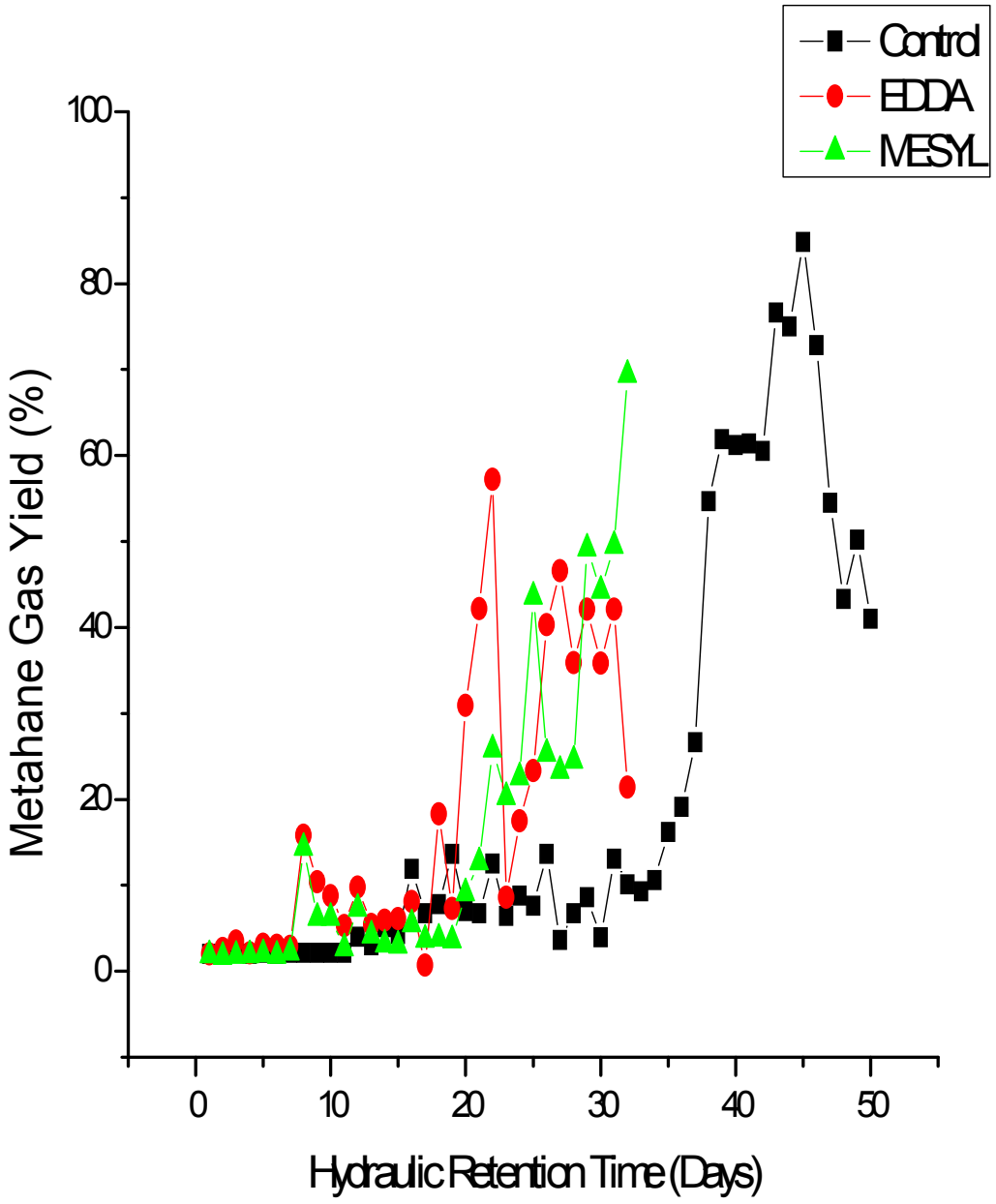


Figure 4.17a: Effect of Addition of Chelating Ligand on HRT in the CH-EDDA and MESYL Digester System

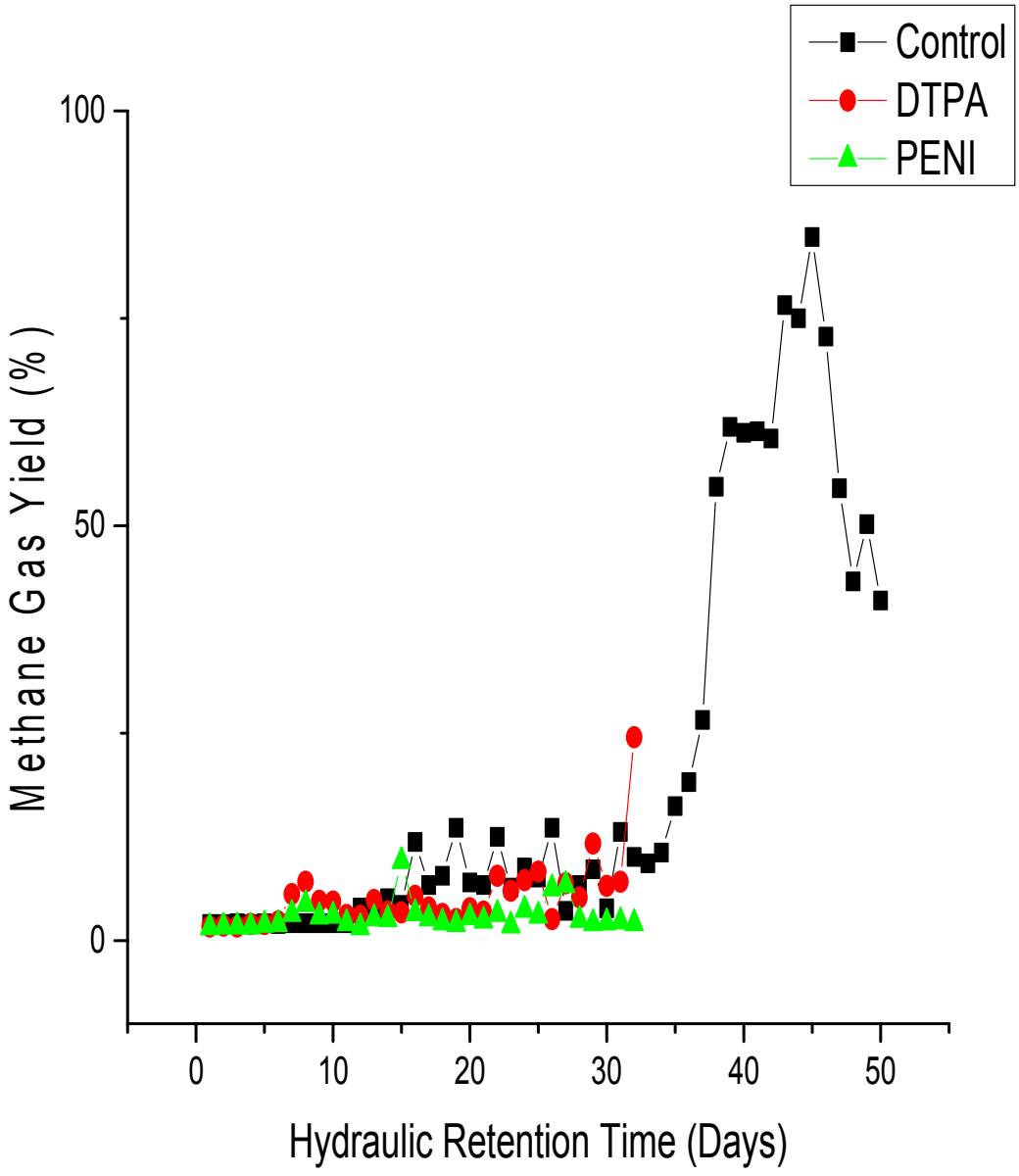


Figure 4.17b: Effect of Addition of Chelating Ligand on HRT in the CH-DTPA and PENI Digester System

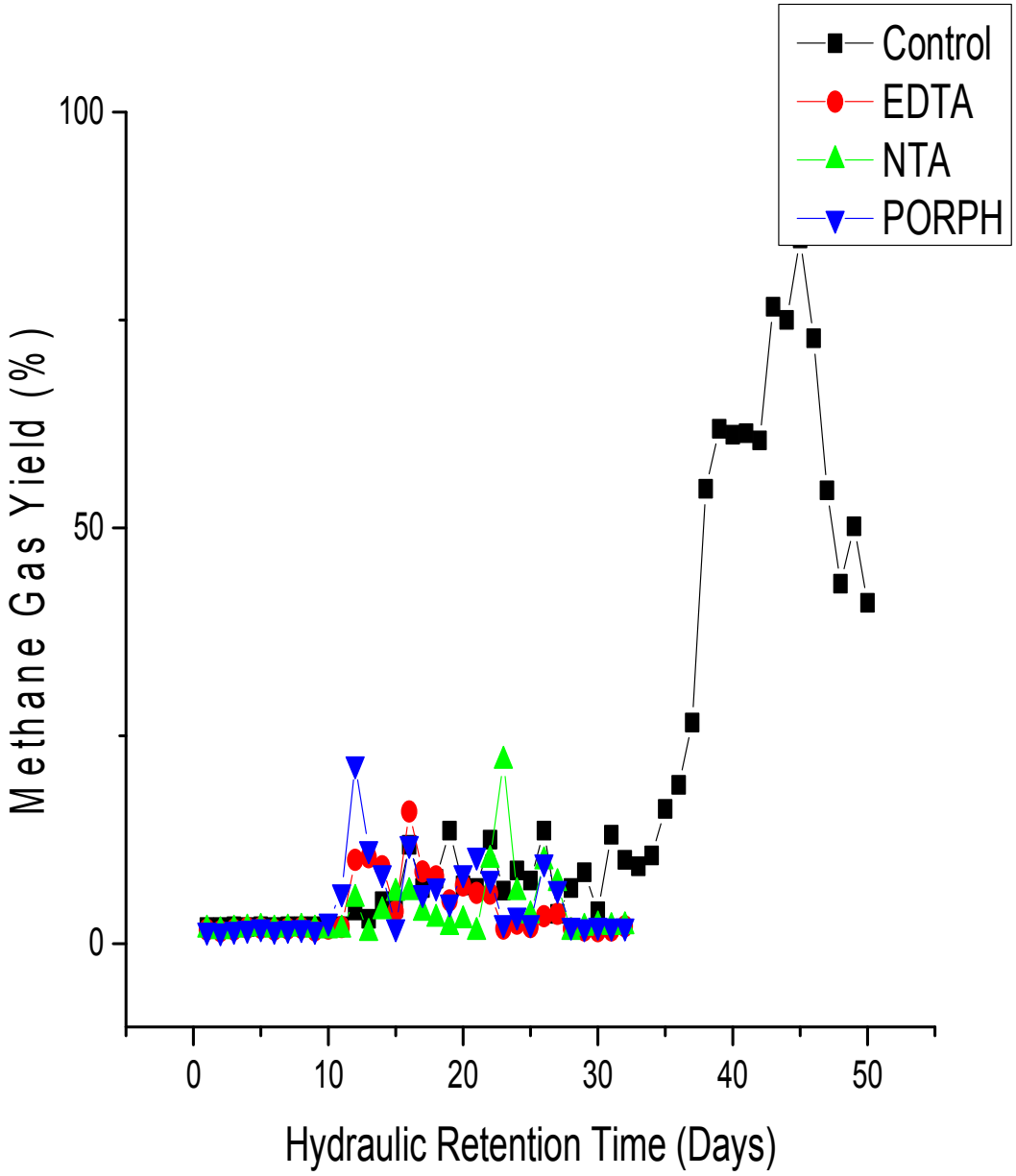


Figure 4.17c: Effect of Addition of Chelating Ligand on HRT in the CH-EDTA, NTA and PORPH Digester System

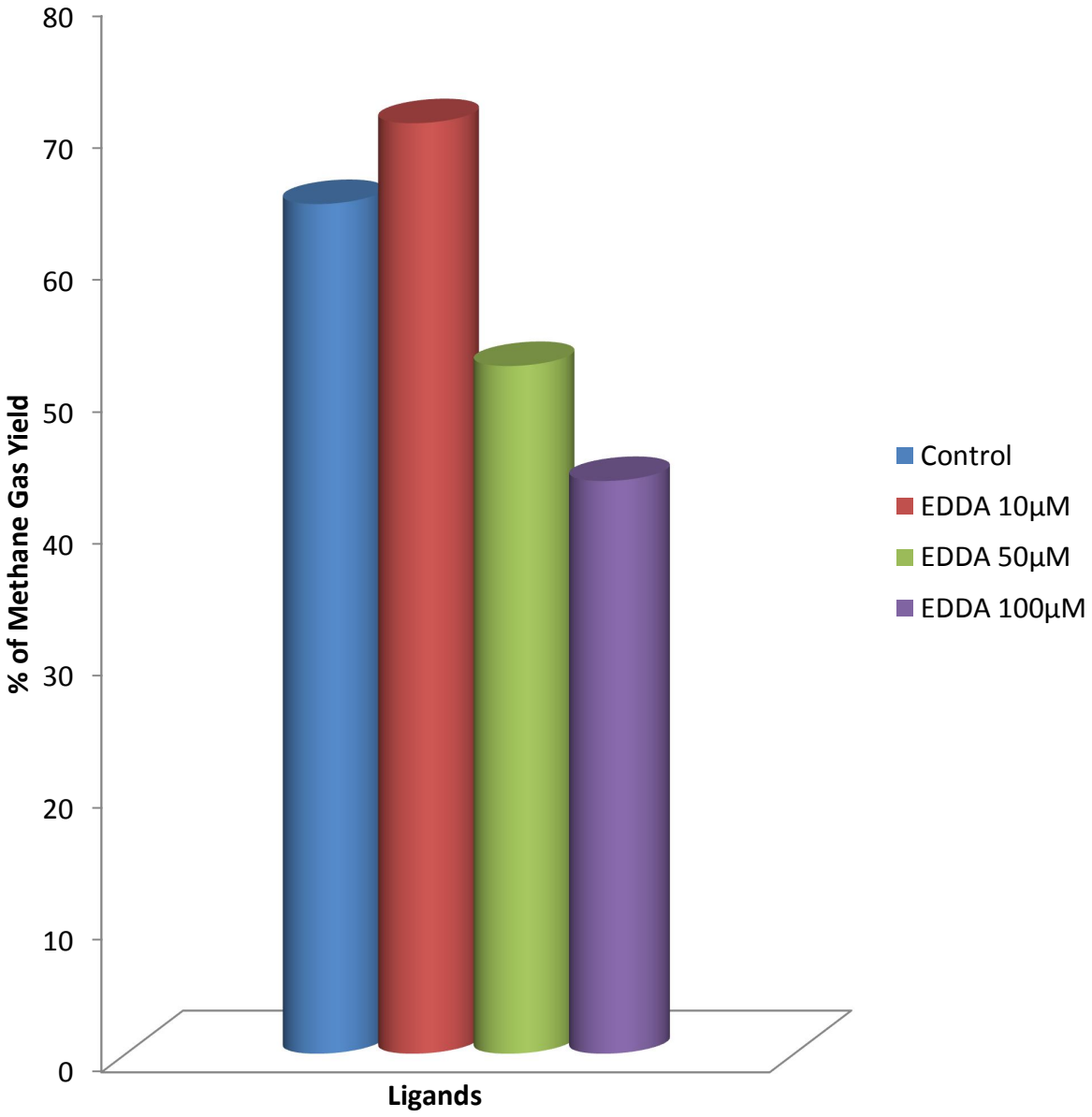


Figure 4.18a: Percentage CH_4 gas yield from EDDA CM Digester System

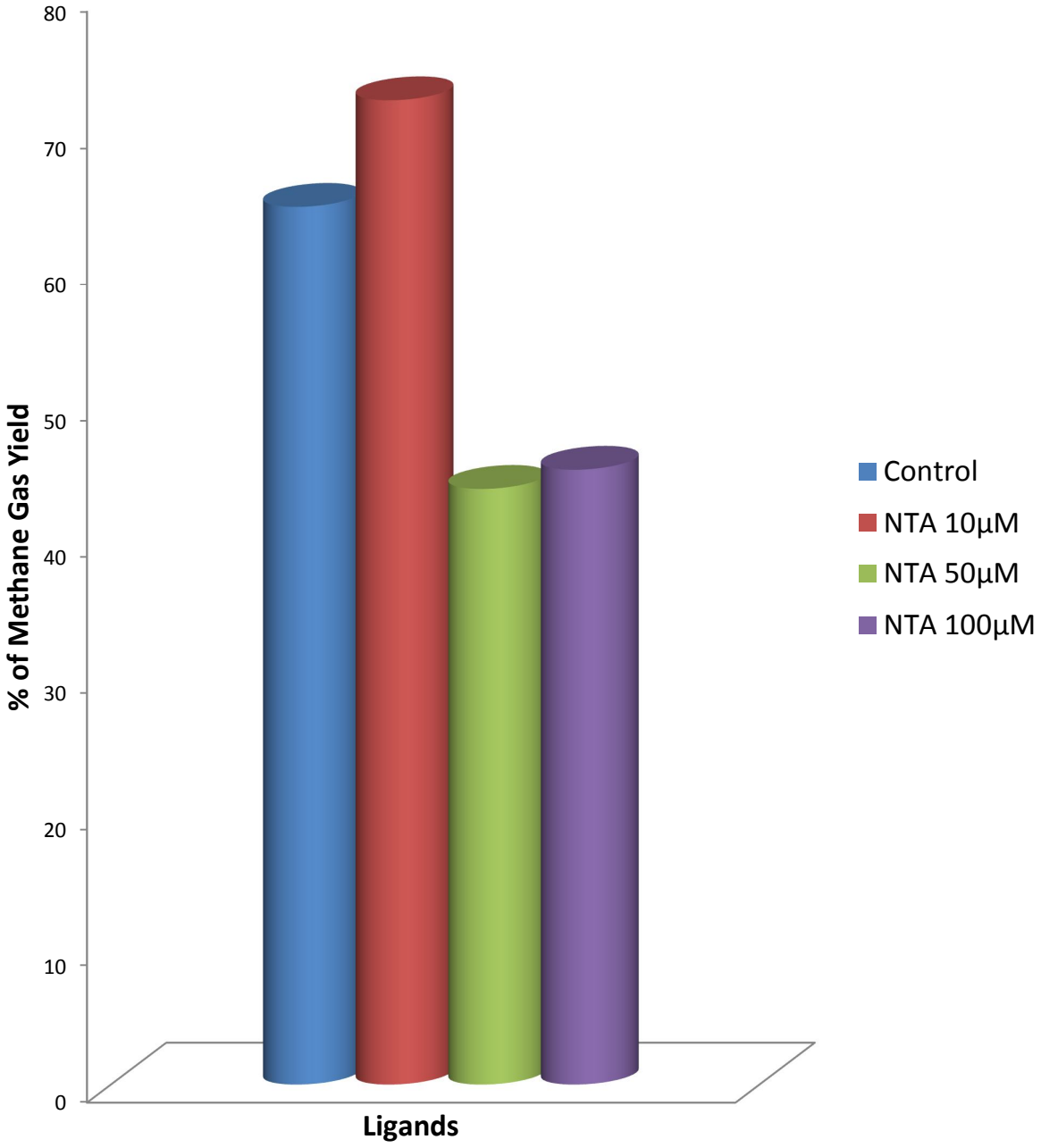


Figure 4.18b: Percentage CH_4 gas yield from NTA CM Digester System

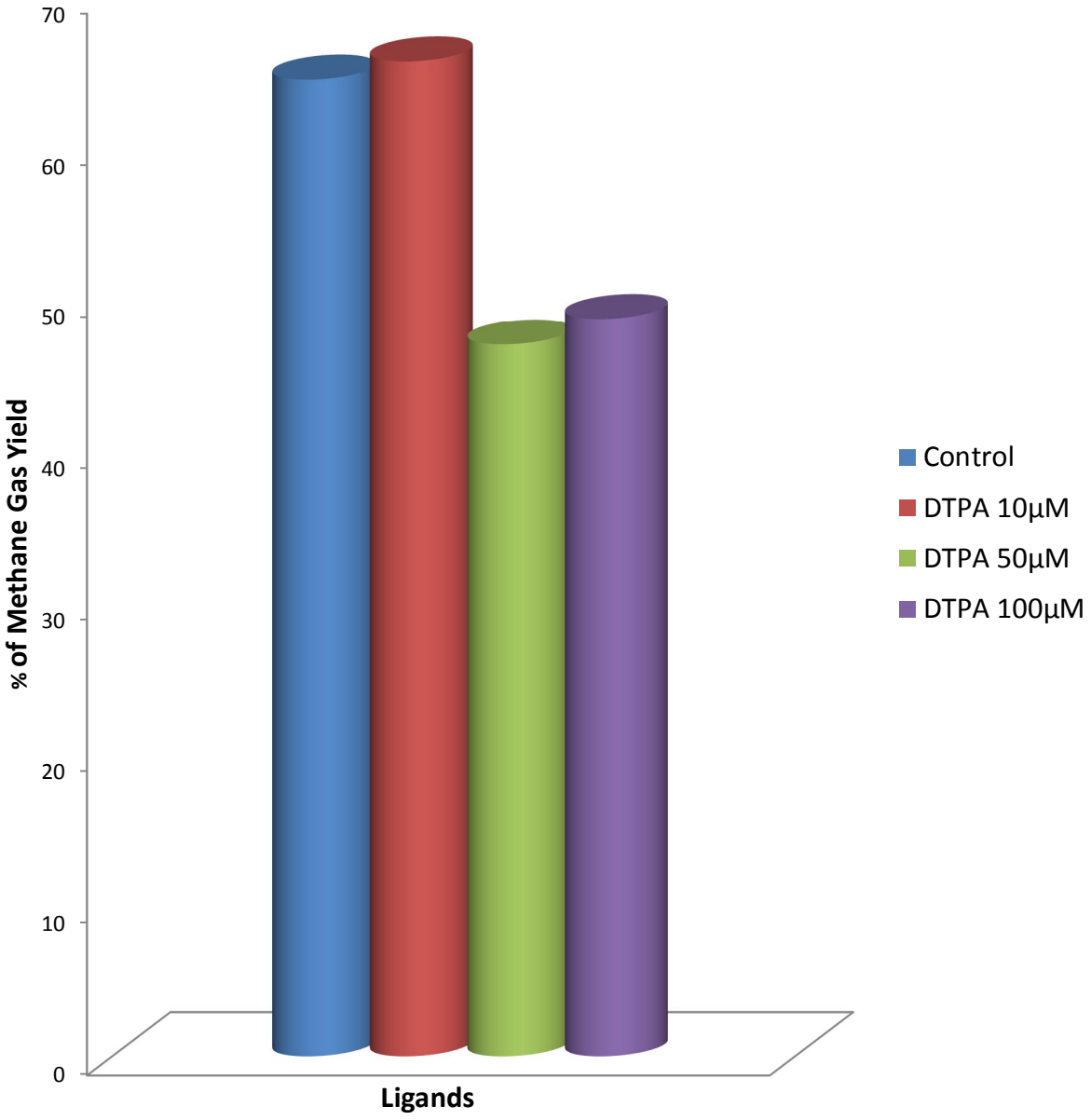


Figure 4.18c: Percentage CH_4 gas yield from DTPA CM Digester System

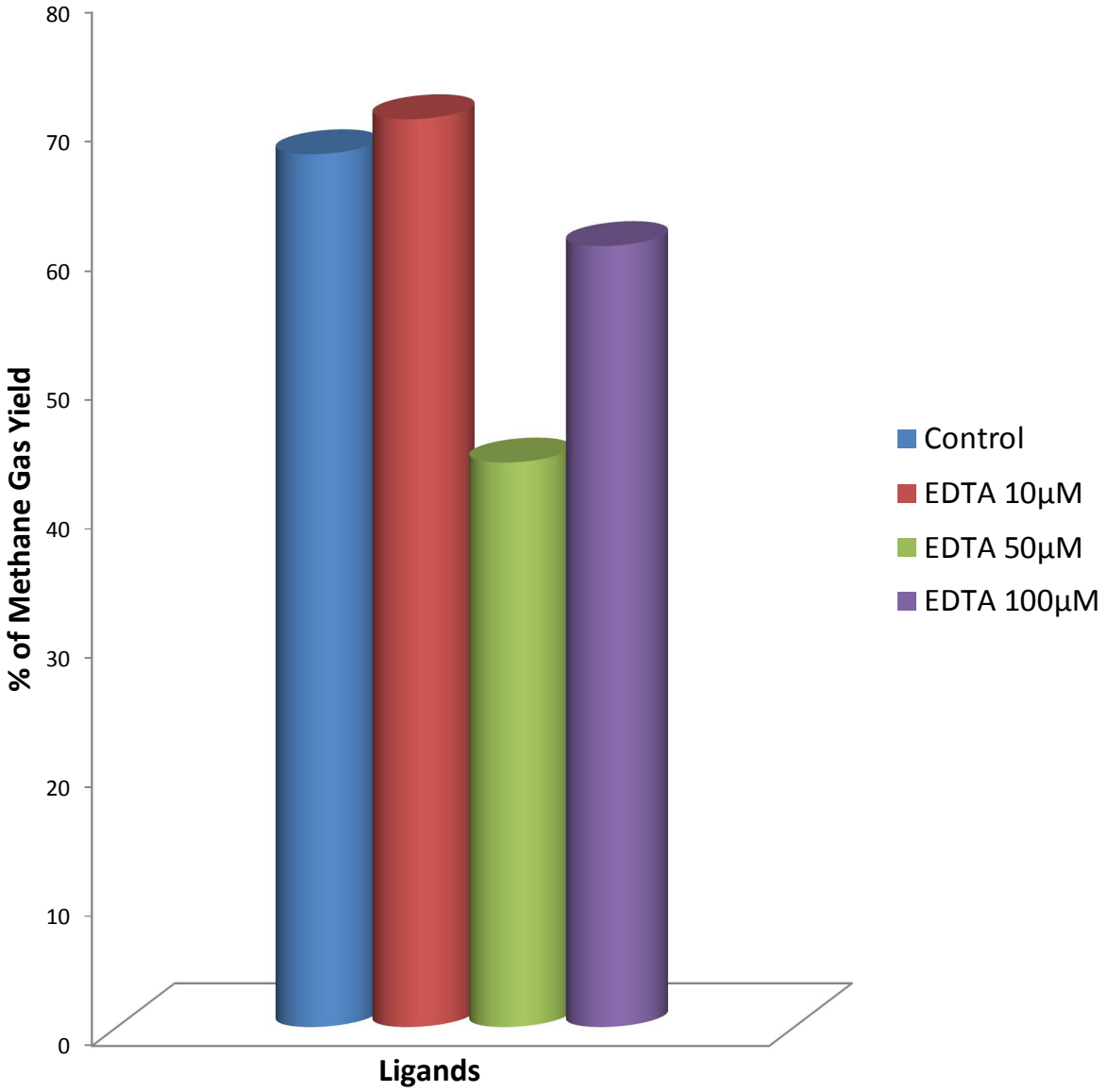


Figure 4.19a: Percentage CH_4 gas yield from EDTA CD Digester System

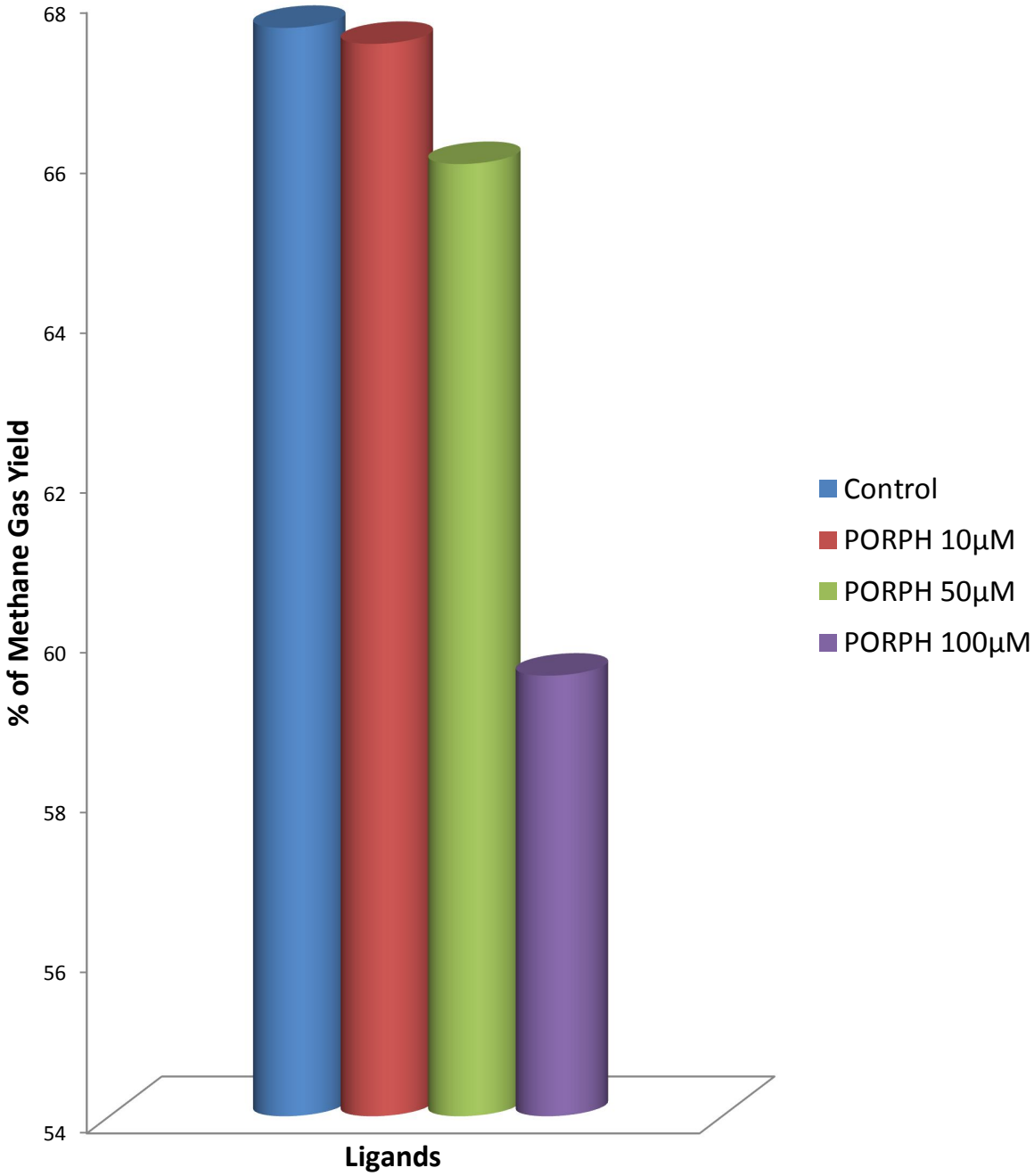


Figure 4.19b: Percentage CH_4 gas yield from PORPH CD Digester System

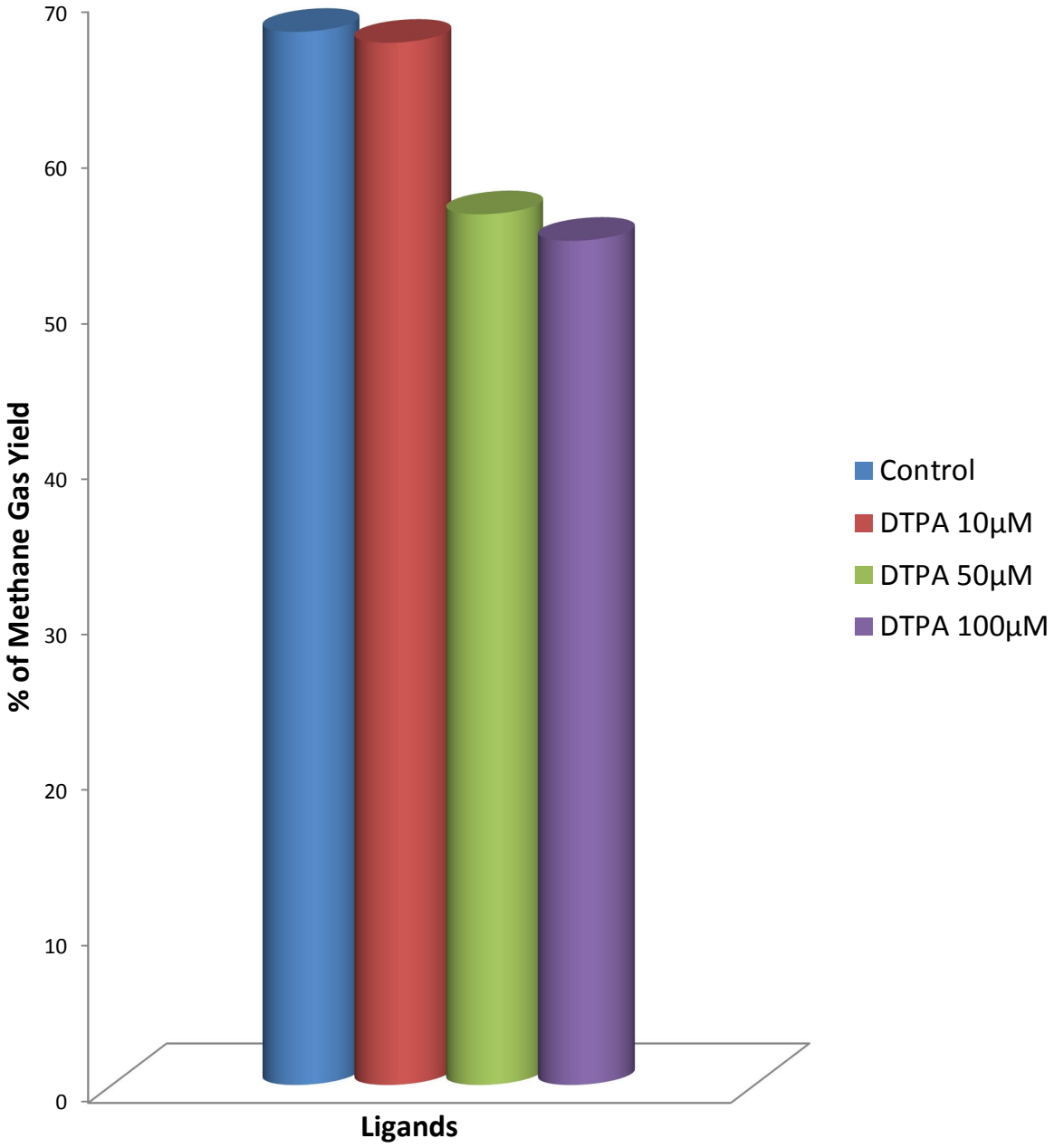


Figure 4.19c: Percentage CH_4 gas yield from DTPA CD Digester System

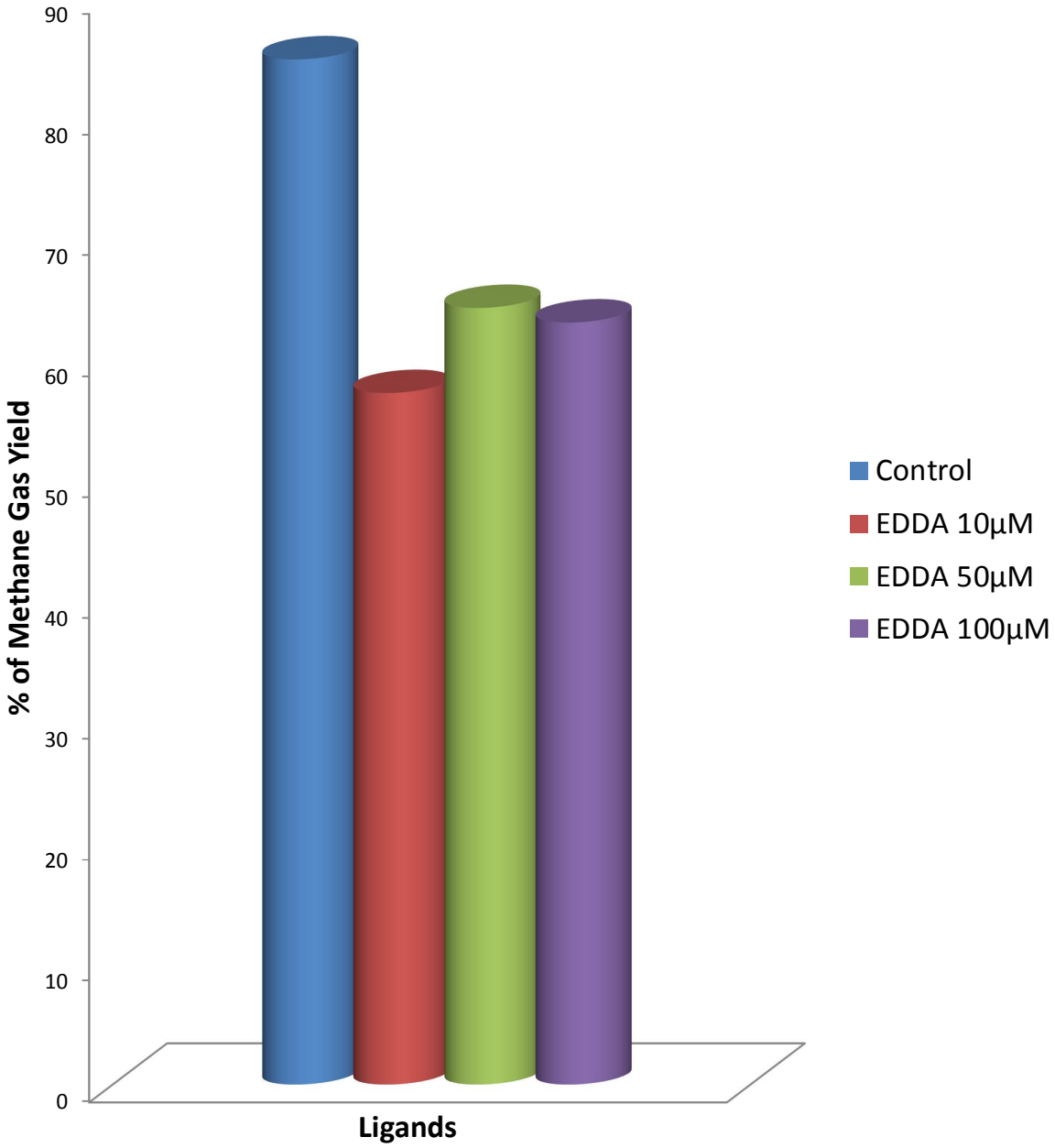


Figure 4.20a: Percentage CH_4 gas yield from EDDA CH Digester System

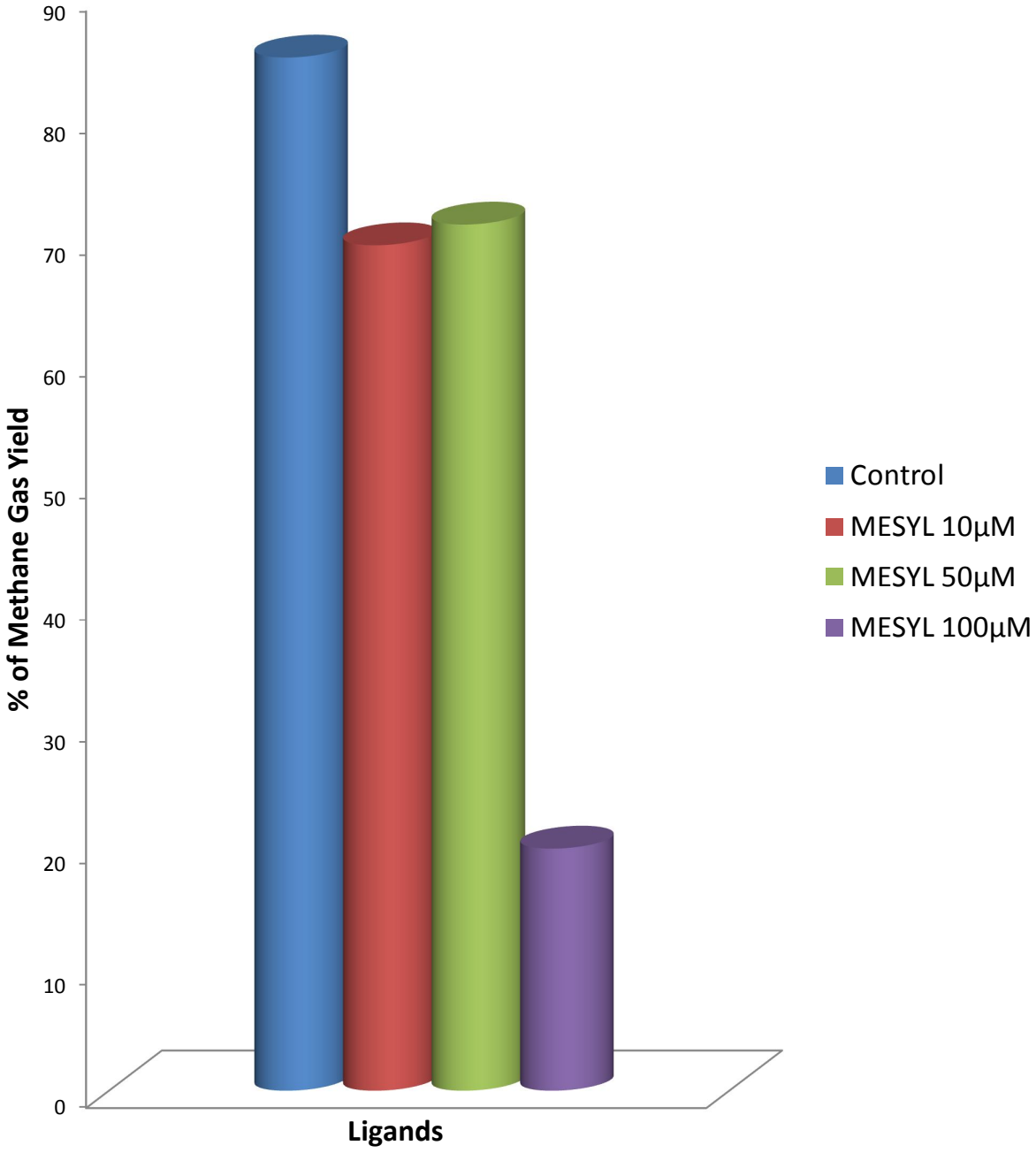


Figure 4.20b: Percentage CH_4 gas yield from MESYL CH Digester System

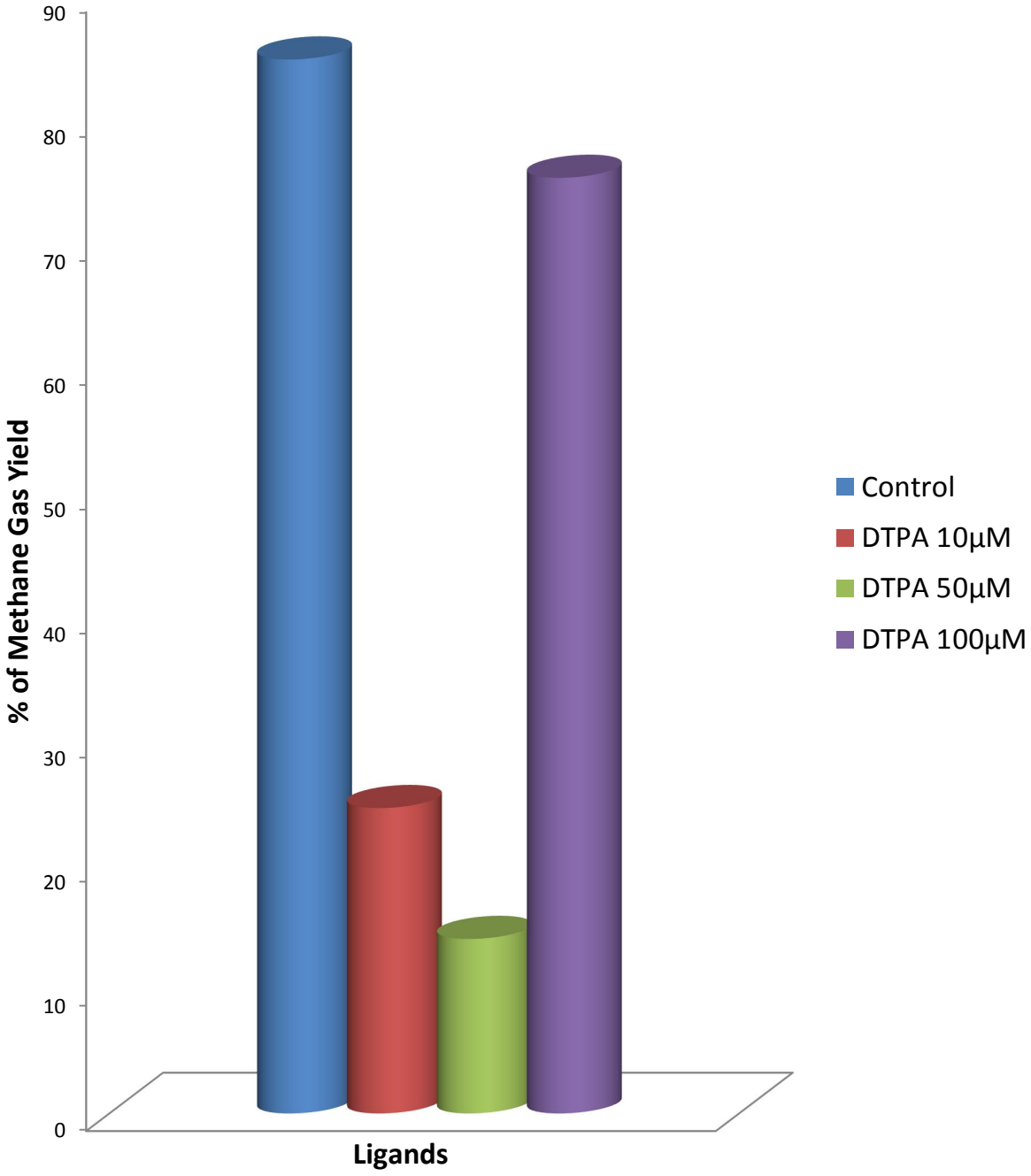


Figure 4.20c: Percentage CH_4 gas yield from DTPA CH Digester System

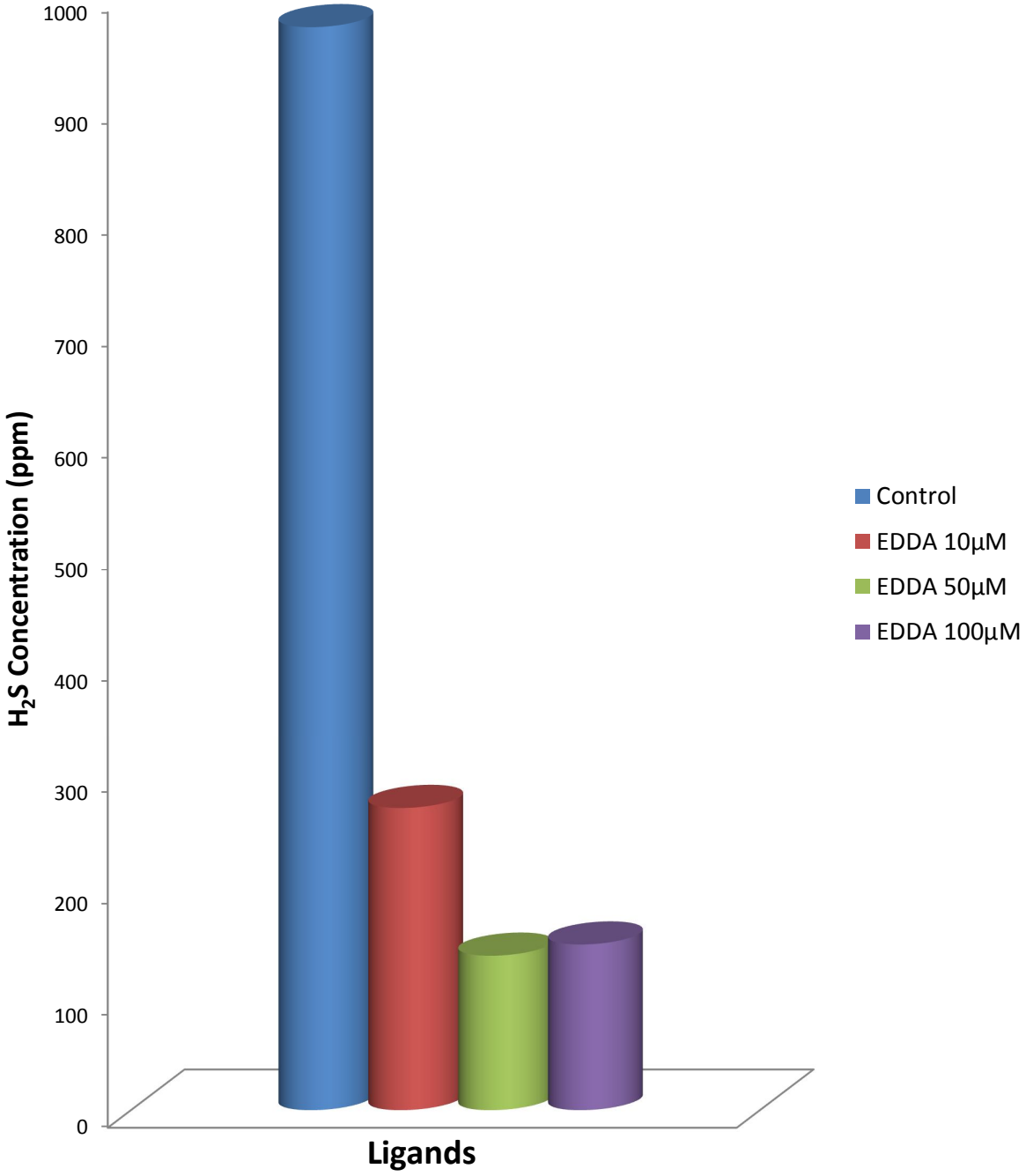


Figure 4.21a: H_2S gas concentration from EDDA CM digester system

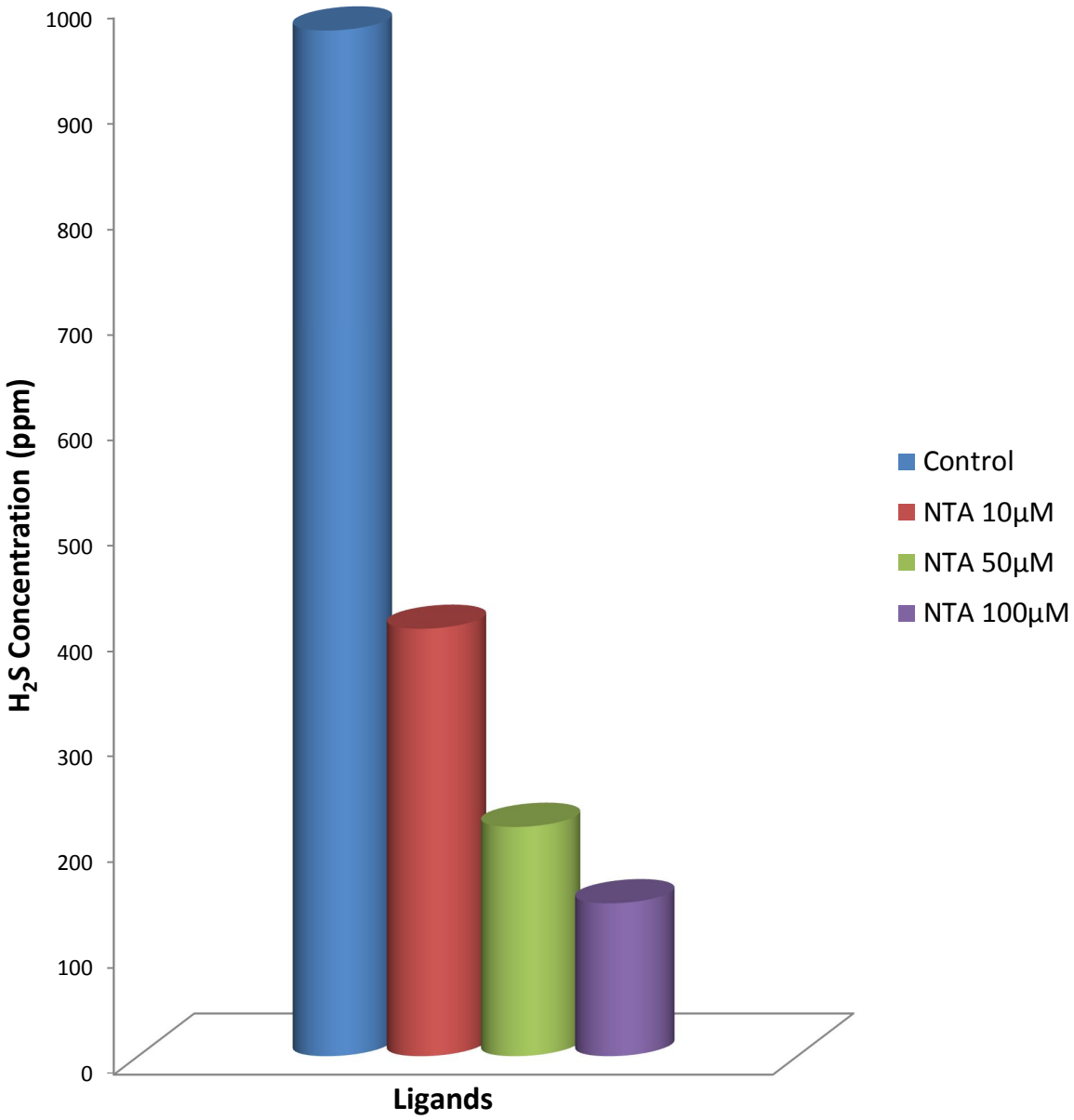


Figure 4.21b: H_2S gas concentration from NTA CM digester system

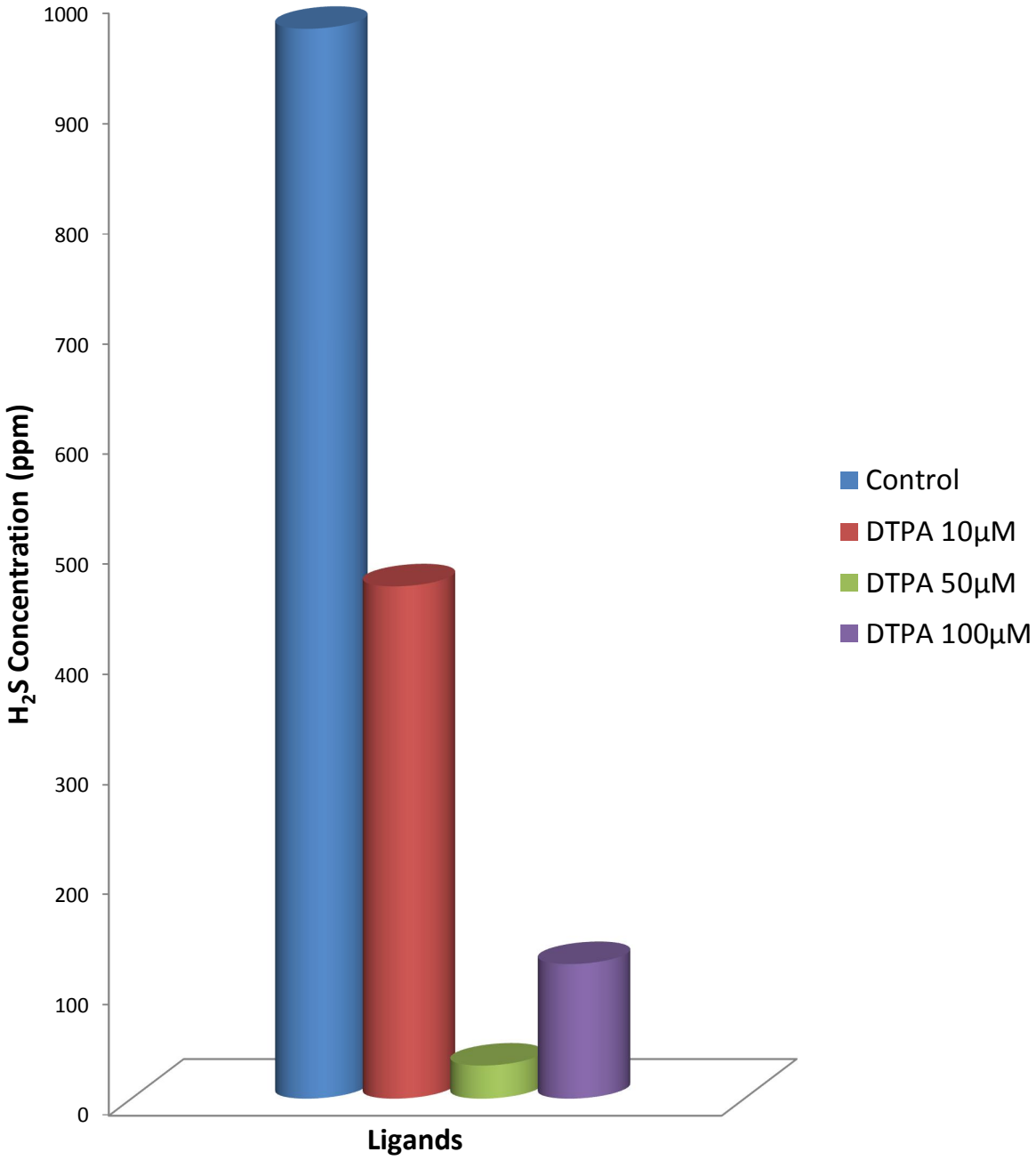


Figure 4.21c: H_2S gas concentration from DTPA CM digester system

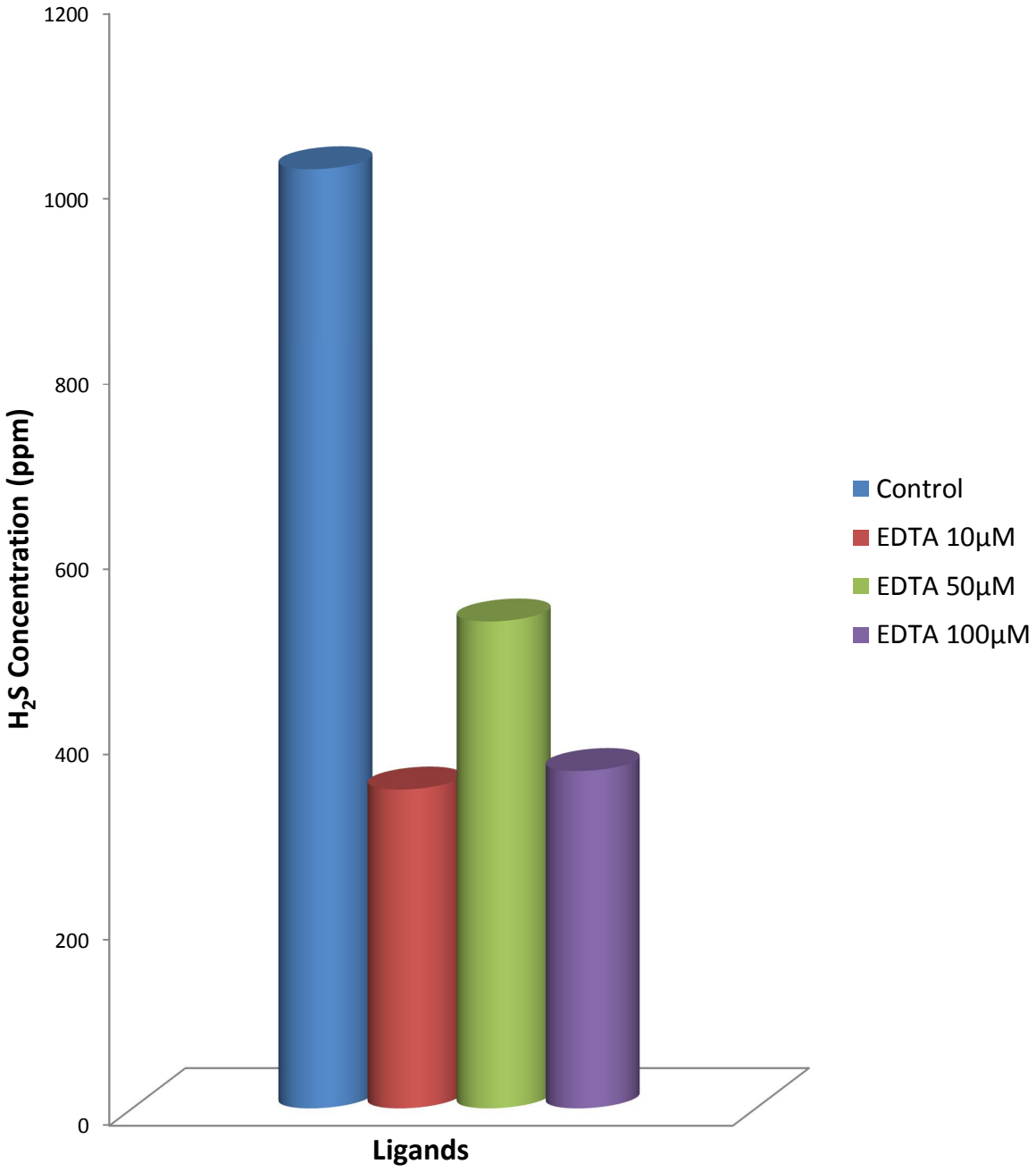


Figure 4.22a: H_2S gas concentration from EDTA CD digester system

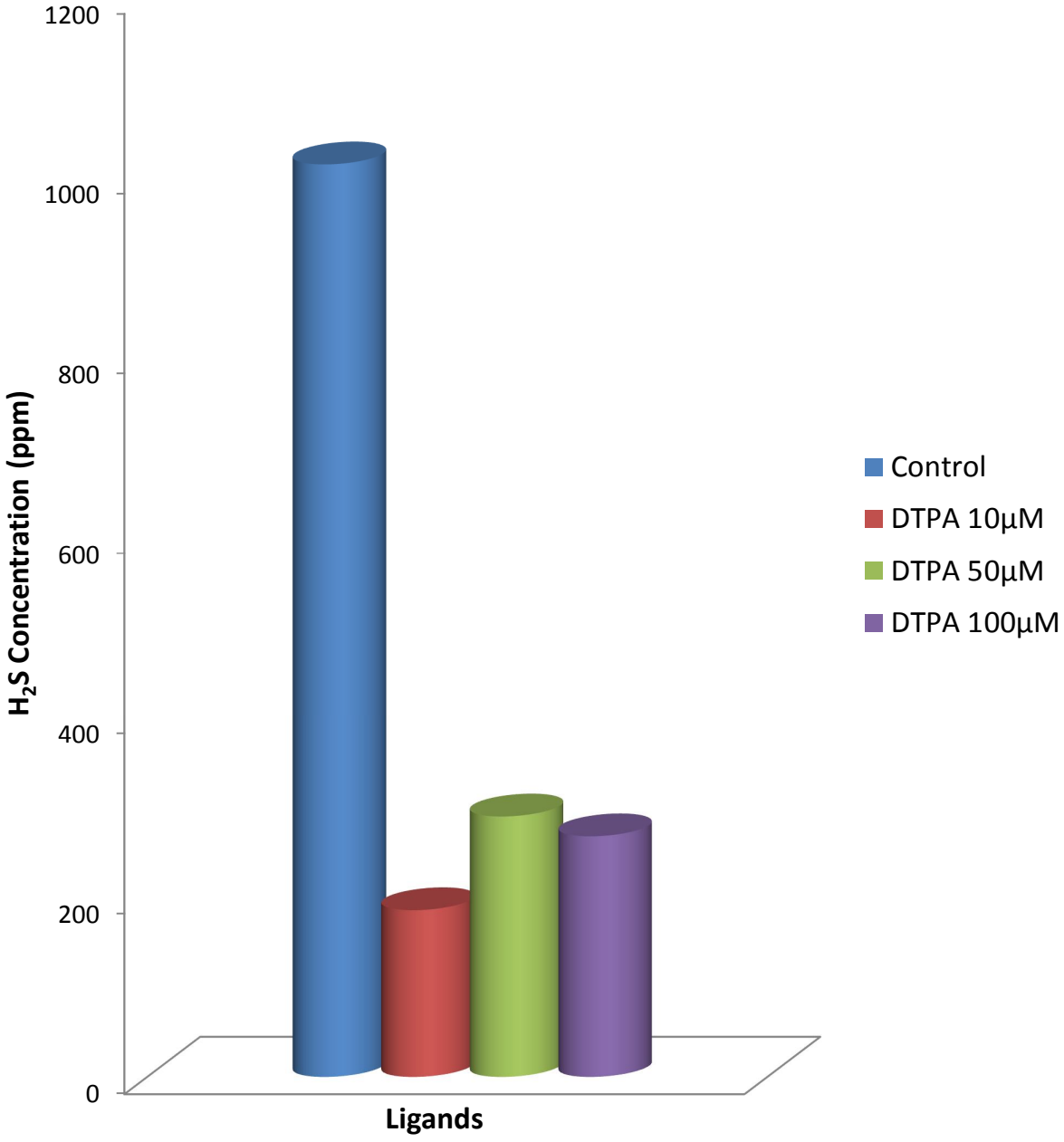


Figure 4.22b: H_2S gas concentration from DTPA CD digester system

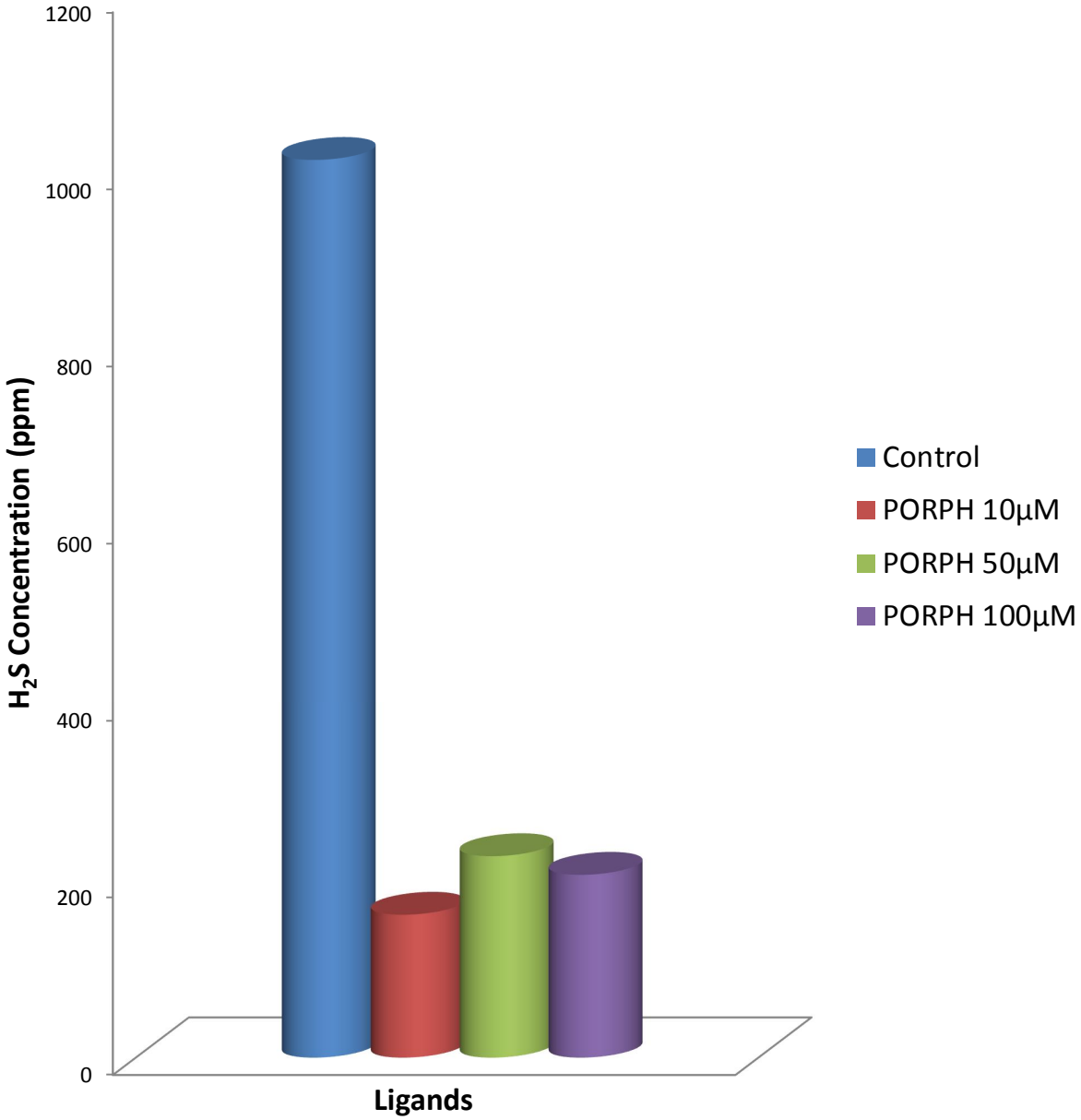


Figure 4.22c: H_2S gas concentration from PORPH CD digester system

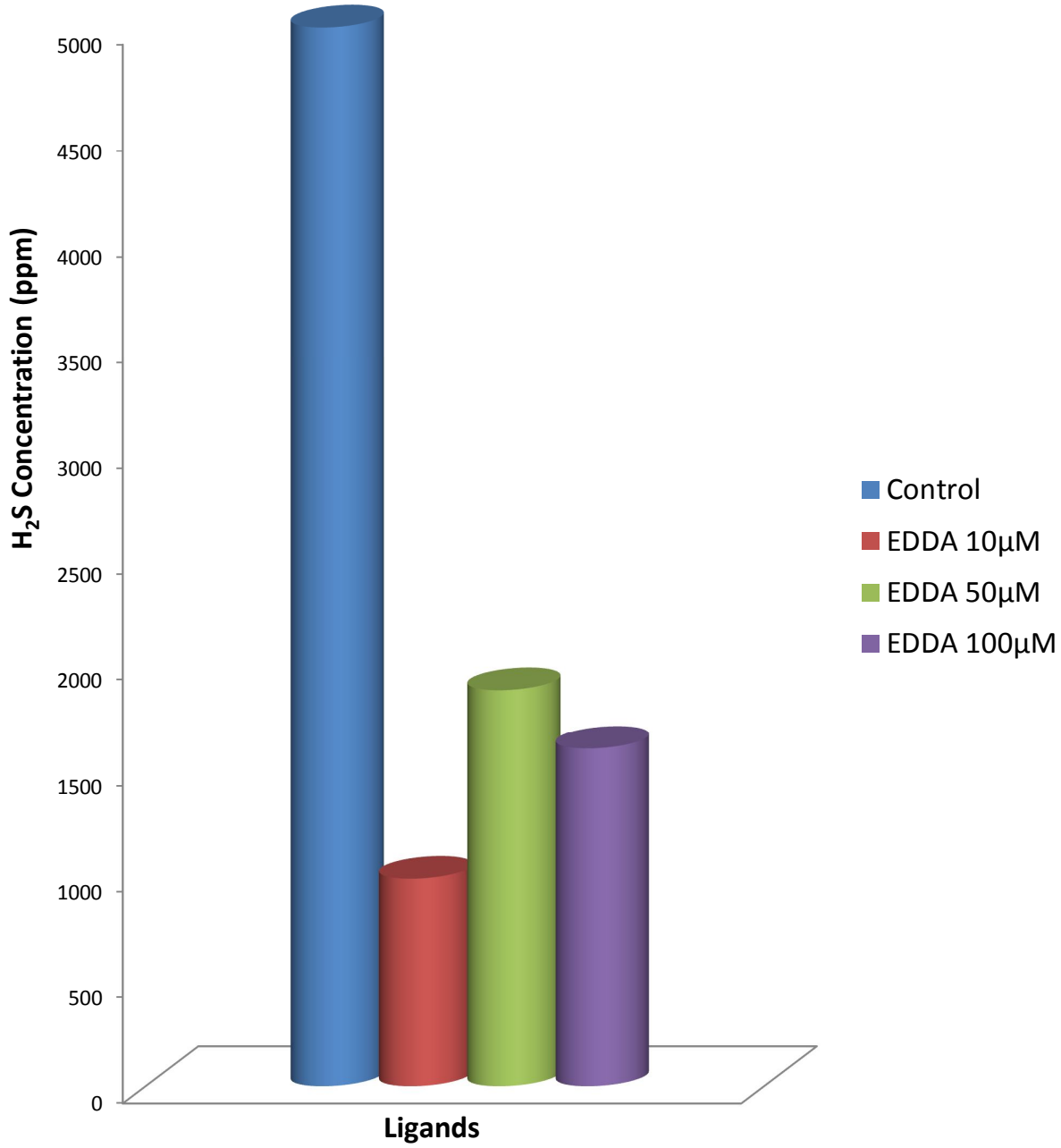


Figure 4.23a: H_2S gas concentration from EDDA CH digester system

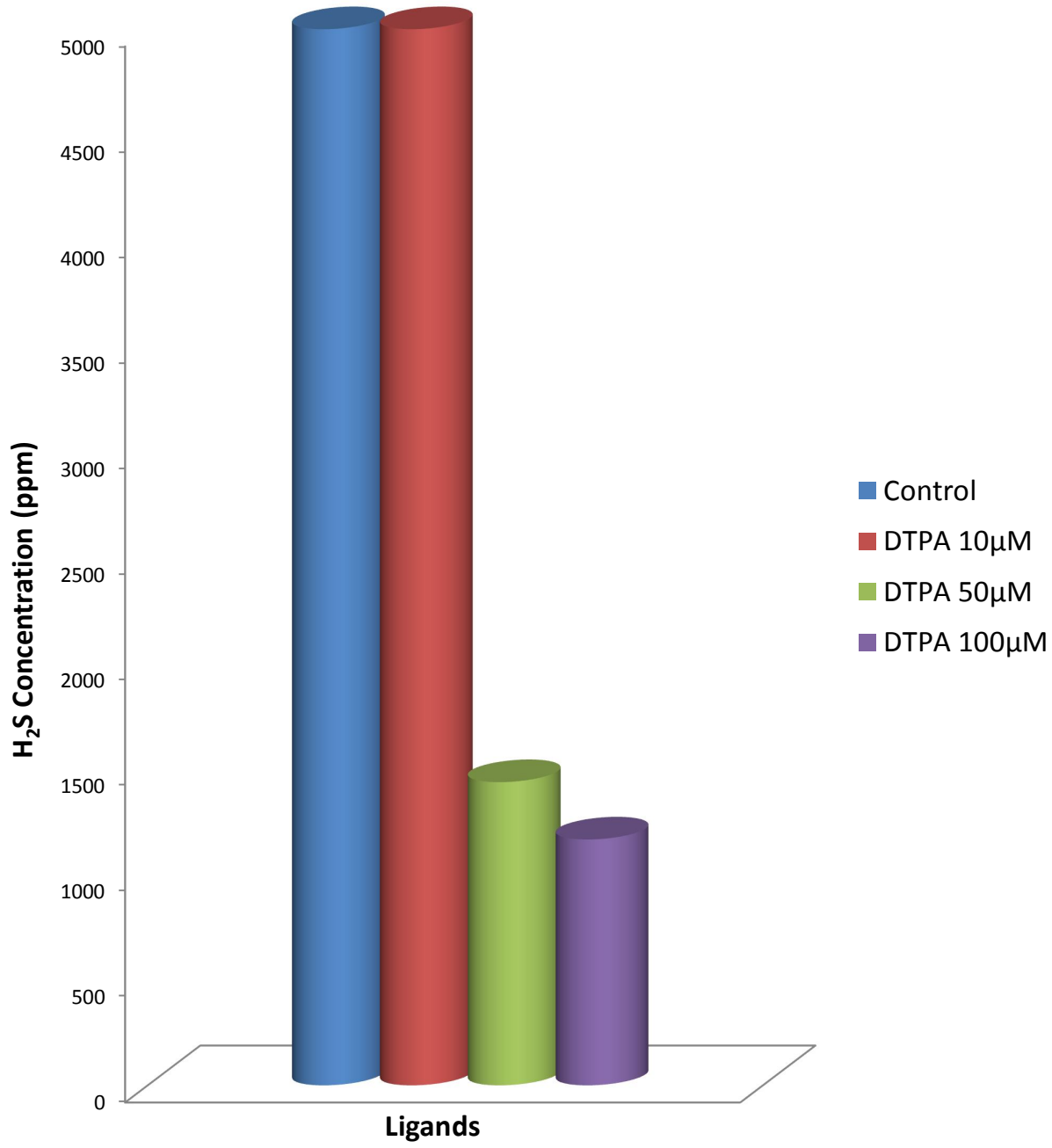


Figure 4.23b: H_2S gas concentration from DTPA CH digester system

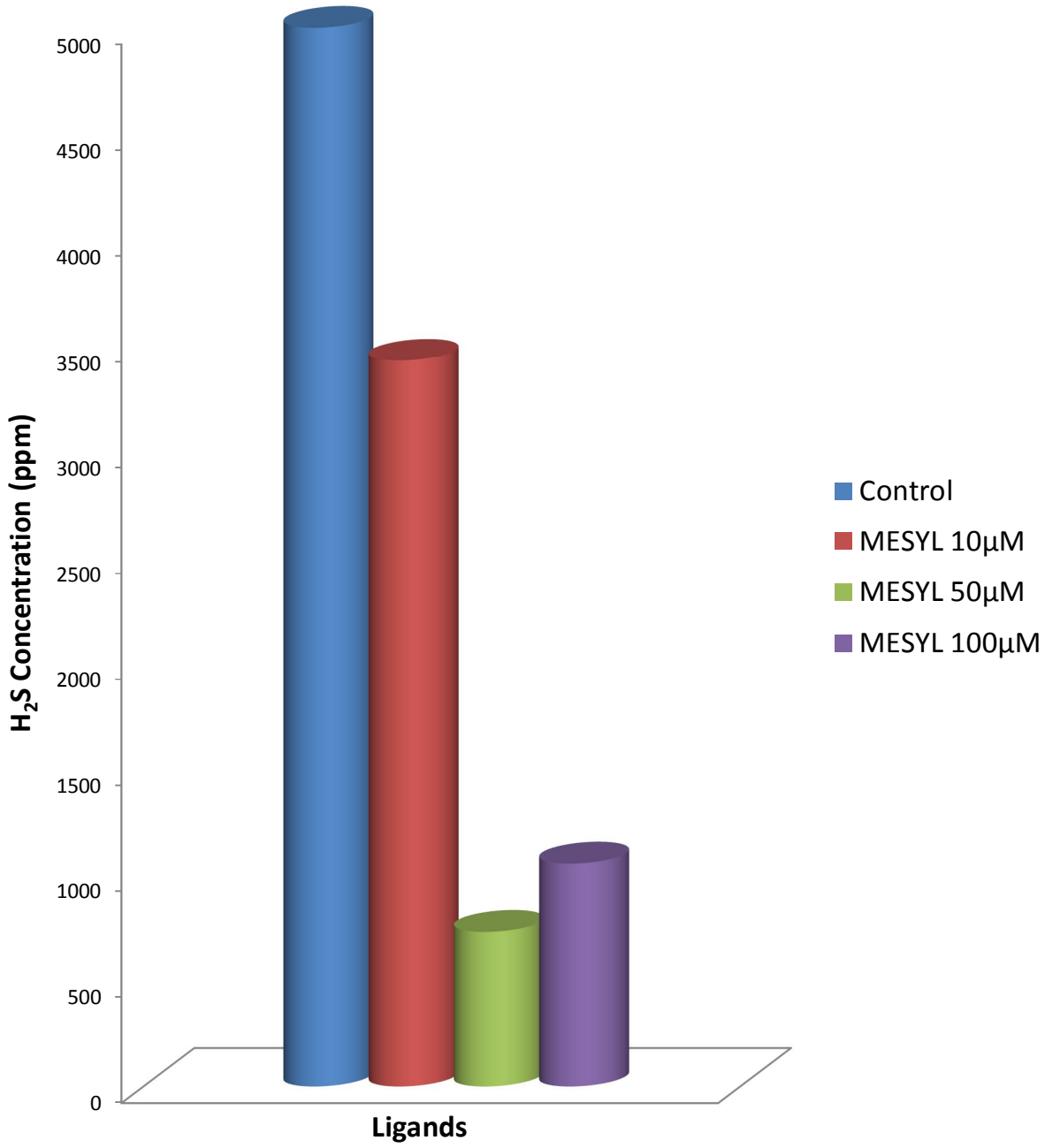


Figure 4.23c: H_2S gas concentration from MESYL CH digester system

4.8 Effect of Increasing Chelating Ligand Concentration on VFA

The concentration of VFA generated within the CM, CD and CH digesters on increasing Chelating ligand concentration was also studied and results recorded in Figures 4.24 -.4.26. The Figures 4.24 – 4.26 were obtained by plotting a graph of VFA concentration from digester systems containing chelating ligands expressed in mg/L, against increasing chelating ligand concentration, from a concentration of 10 μM to 100 μM.

4.9 Effect of Increasing Chelating Ligand Concentration on HRT

A study on the effect of increasing concentration of chelating ligands on HRT was equally carried out for the best performing chelating ligand systems i.e. EDDA, NTA, and DTPA for the CM digester system; EDTA, DTPA and PORPH for the CD digester system and lastly, EDDA, DTPA and MESYL for the CH digester system. Figures 4.27 – 4.35 shows the plot of percentage CH_4 gas Yield generated at each digester system on increasing chelating ligand concentration; from a concentration of 10 μM to 100 μM against Time expressed in days.

4.10 Co-digestion

The process of co-digestion involves mixing of different types of feed stocks before digestion to control the C/N ratio (Naomichi and Yutaka, 2007). The effect of the process was studied for six different substrate blends labelled CH: CM, CH: GU, GU: BL, CH: BL, CM: BL and CM: GU; which were operated over a period of 35 days at mesophilic temperature range of 29 - 31°C. Figure 4.36 shows the performance recorded (i.e. percentage CH_4 gas yield) in each digester system, plotted against the six substrate blends.

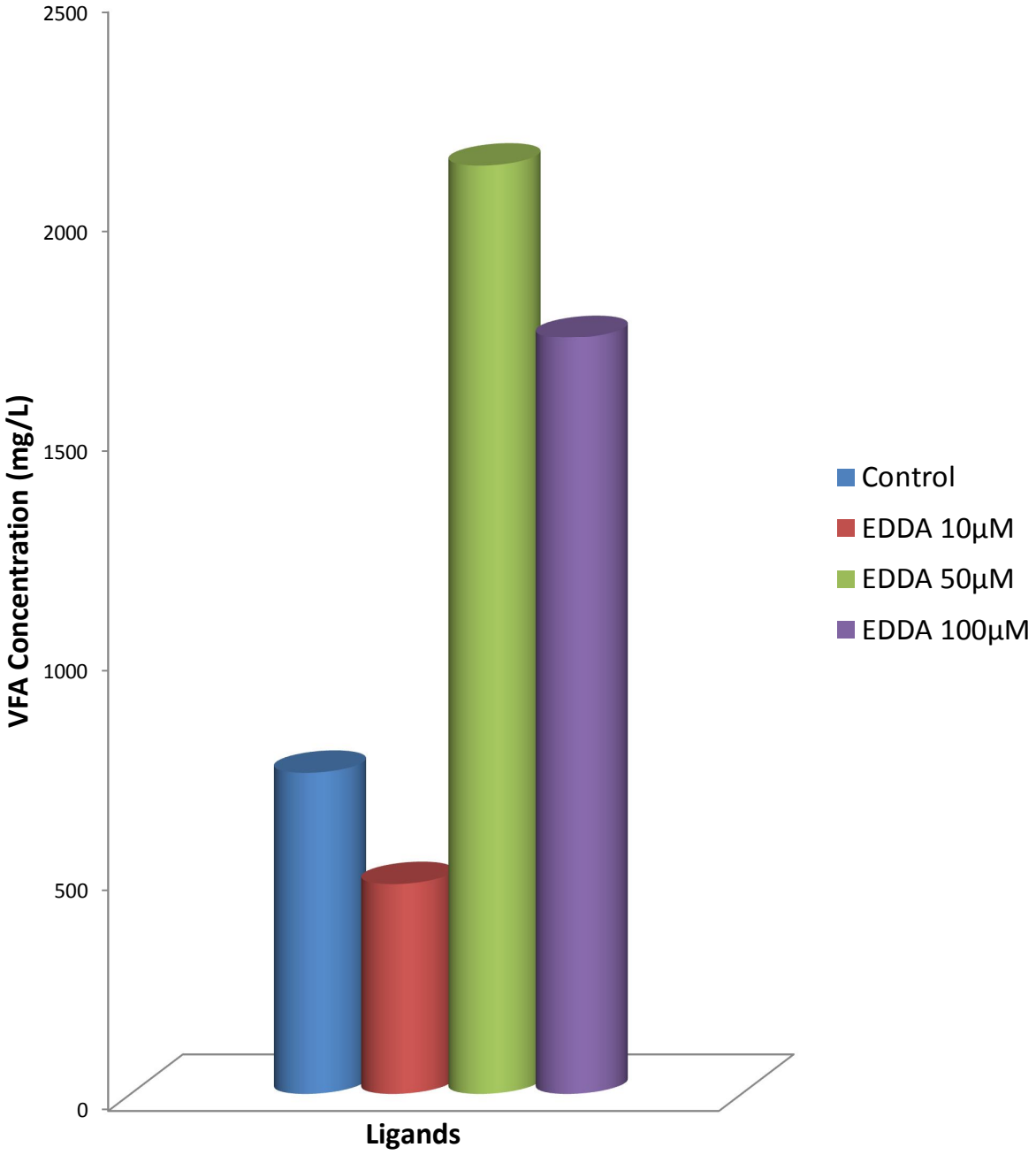


Figure 4.24a: Concentration of VFA in CM EDDA Digester

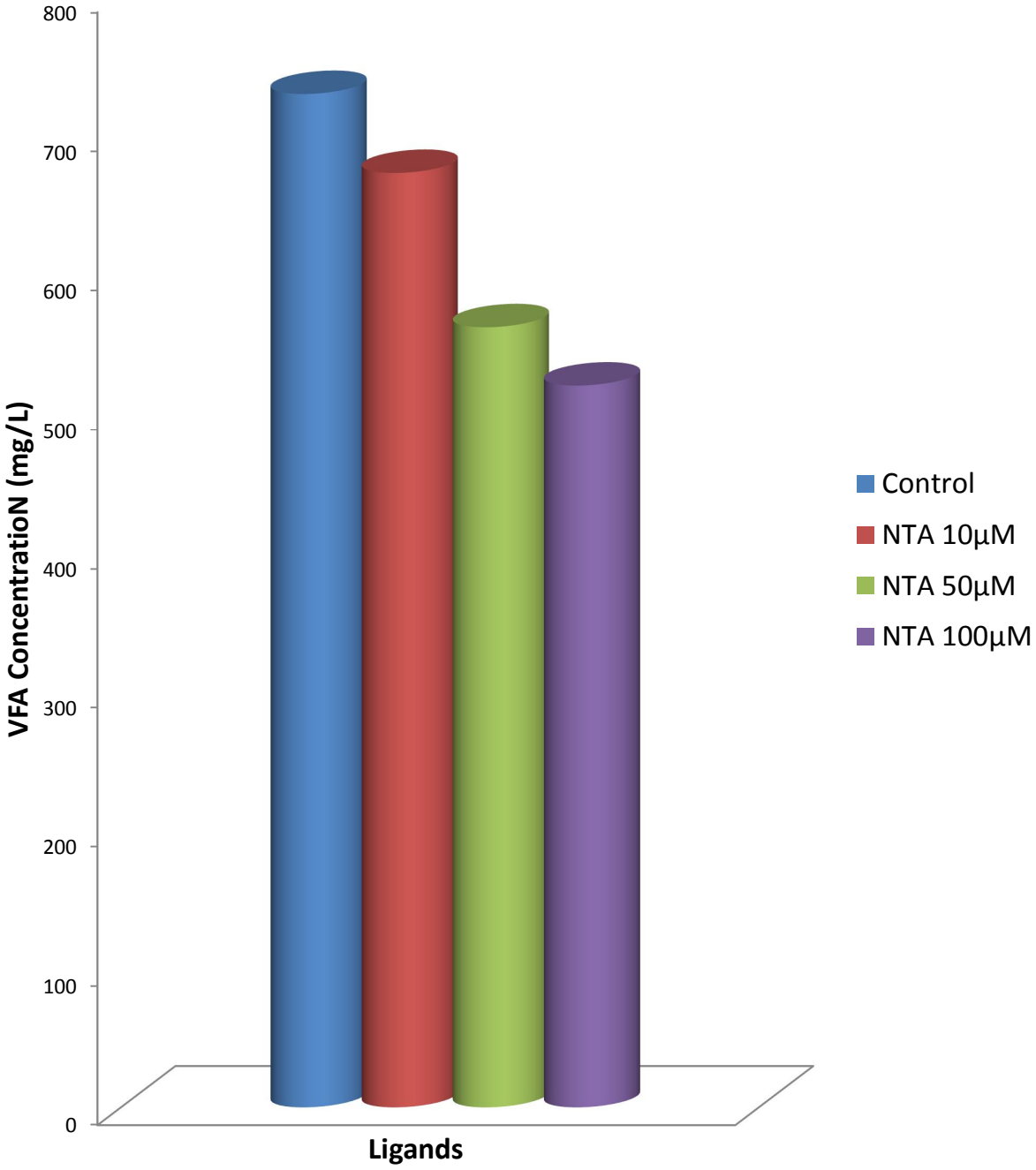


Figure 4.24b: Concentration of VFA in CM NTA Digester

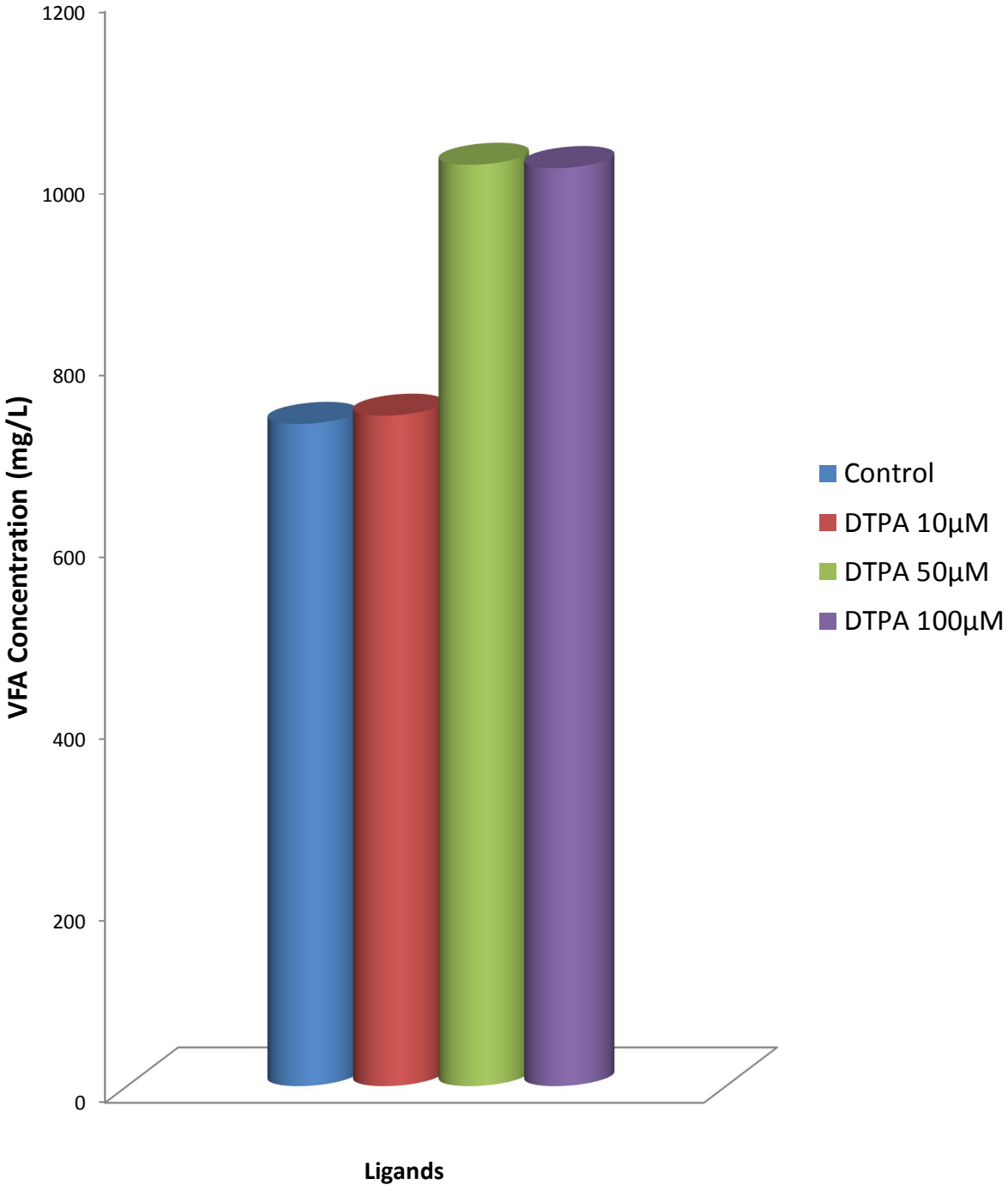


Figure 4.24c: Concentration of VFA in CM DTPA Digester

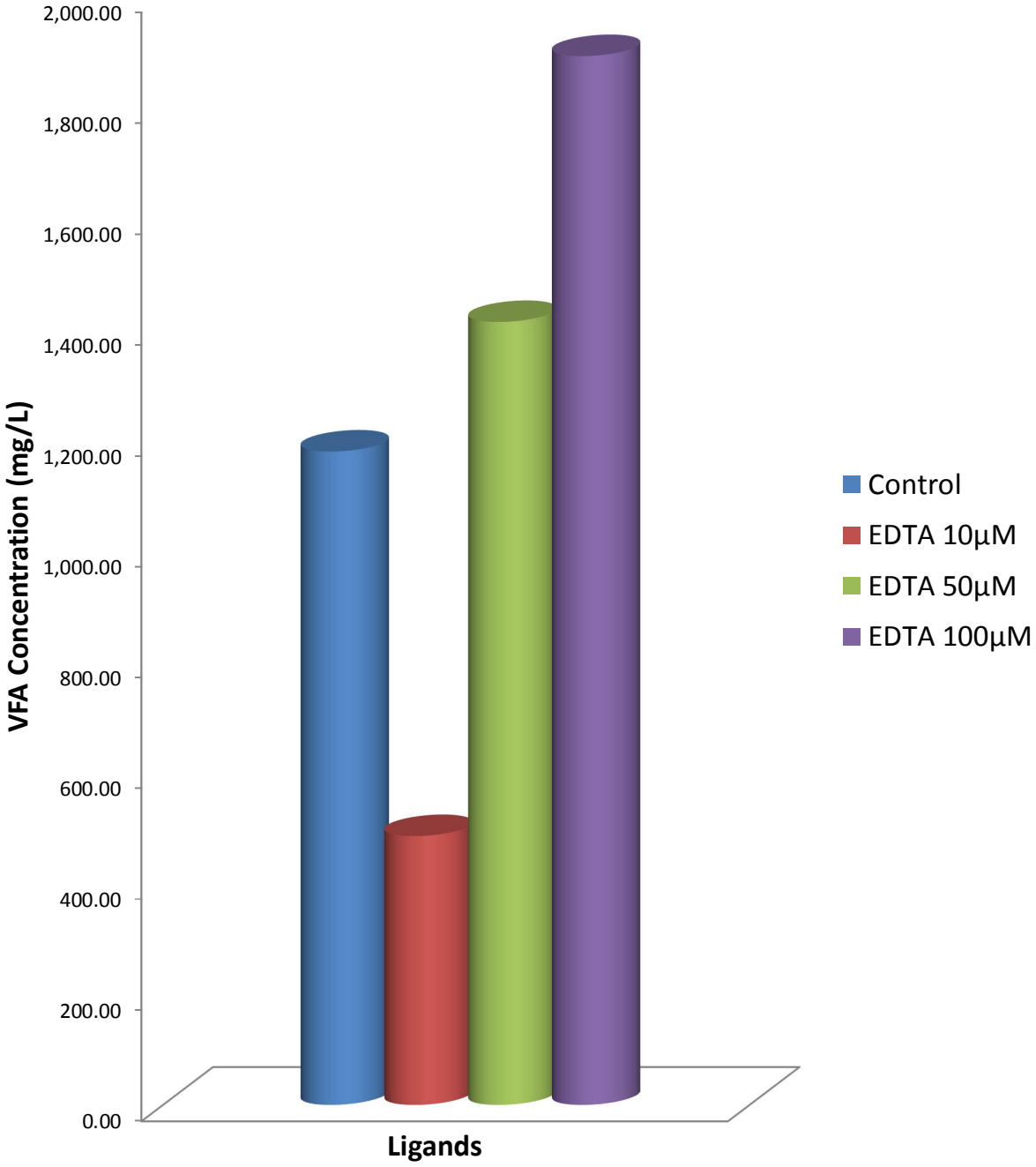


Figure 4.25a: Concentration of VFA in CD EDTA Digester

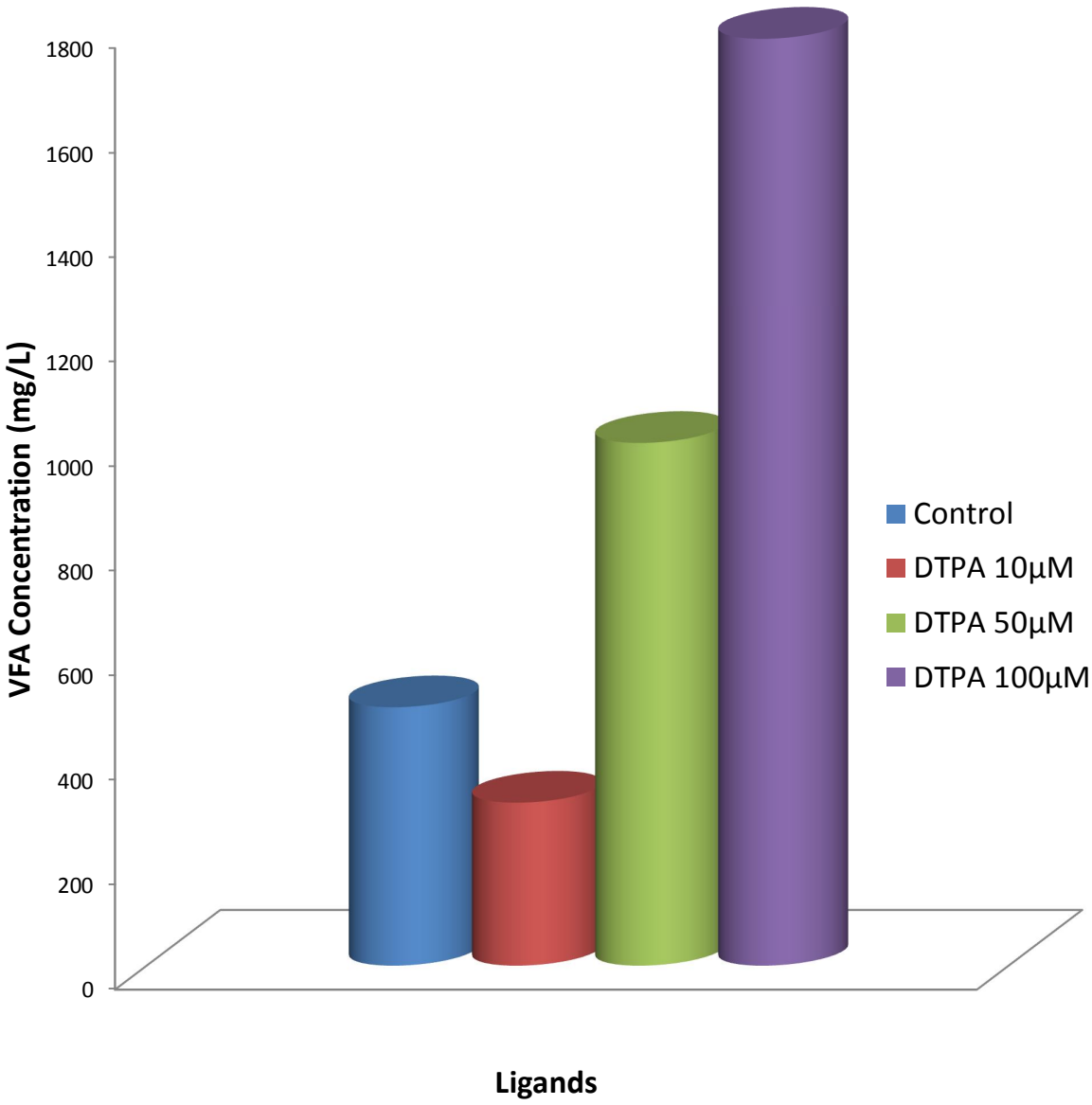


Figure 4.25b: Concentration of VFA in CD DTPA Digester

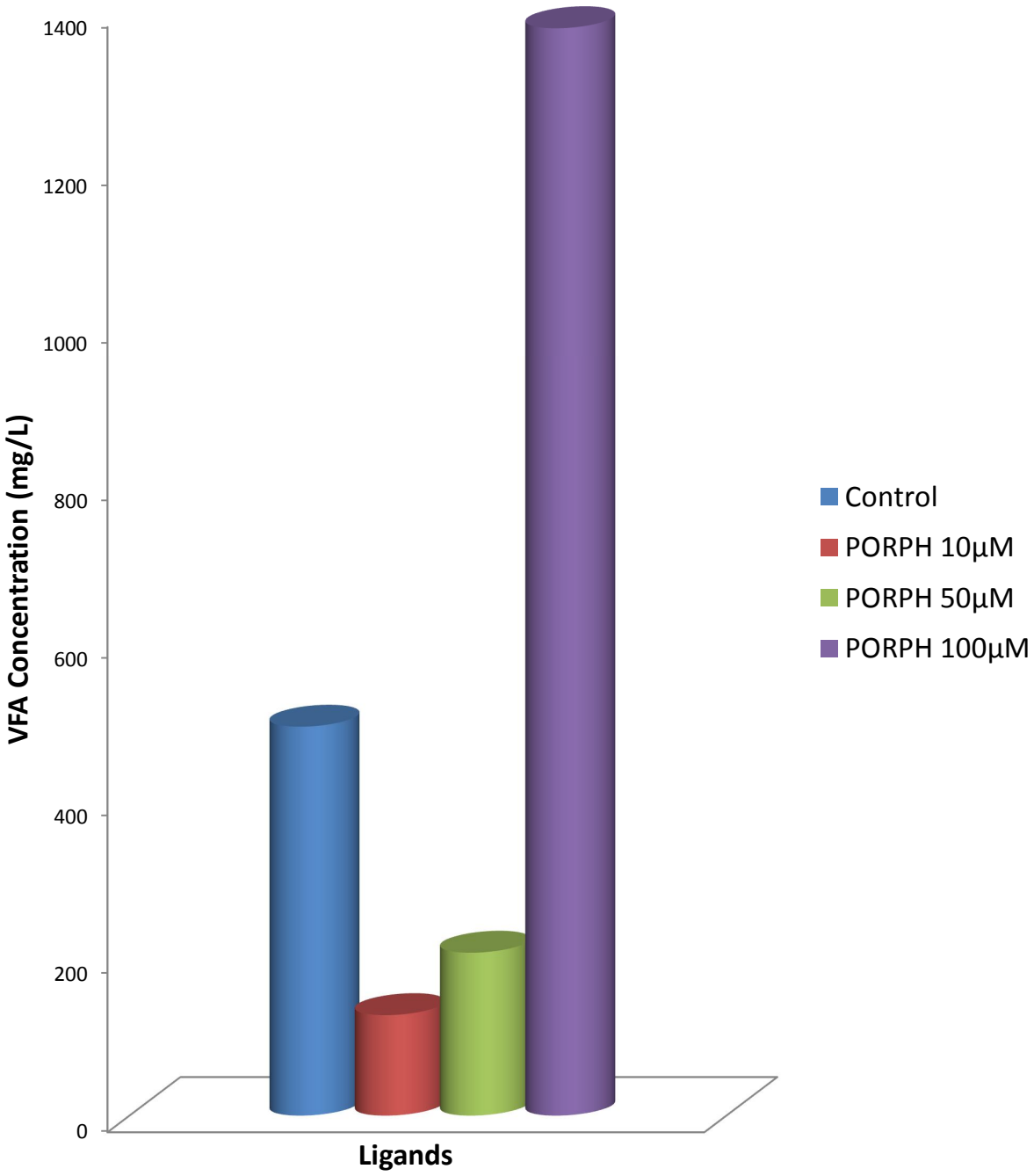


Figure 4.25c: Concentration of VFA in CD PORPH Digester

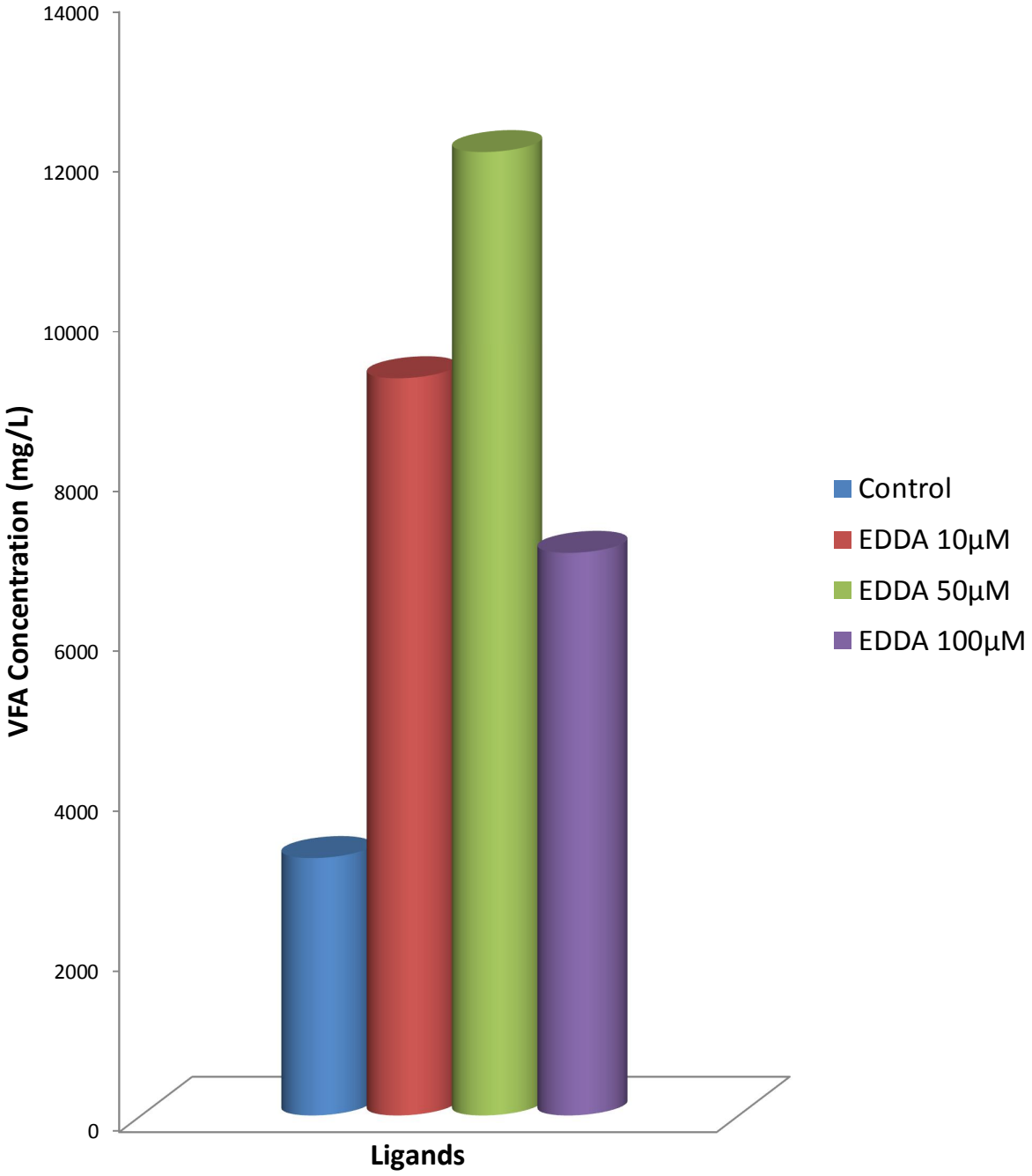


Figure 4.26a: Concentration of VFA in CH EDDA Digester

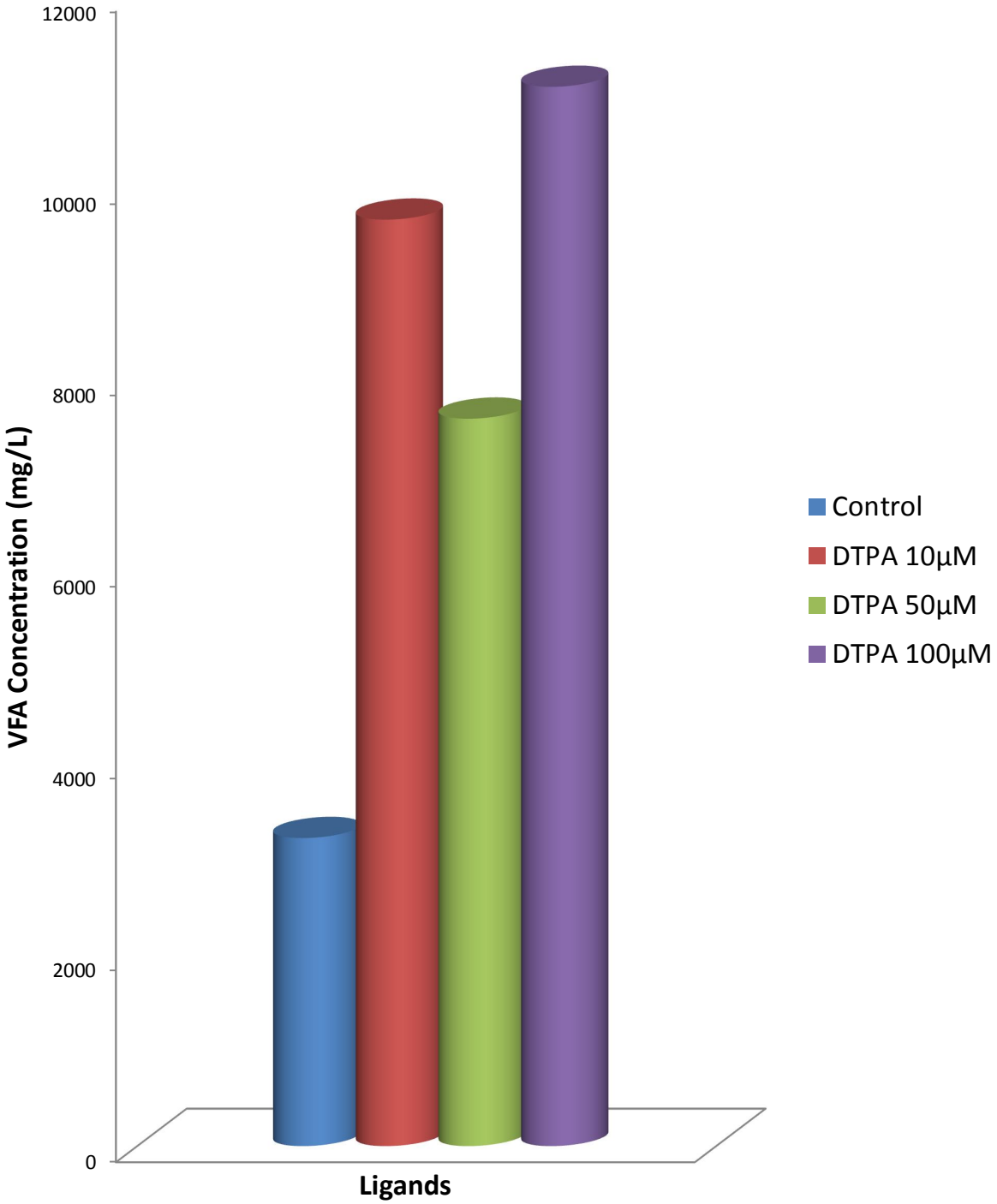


Figure 4.26b: Concentration of VFA in CH DTPA Digester

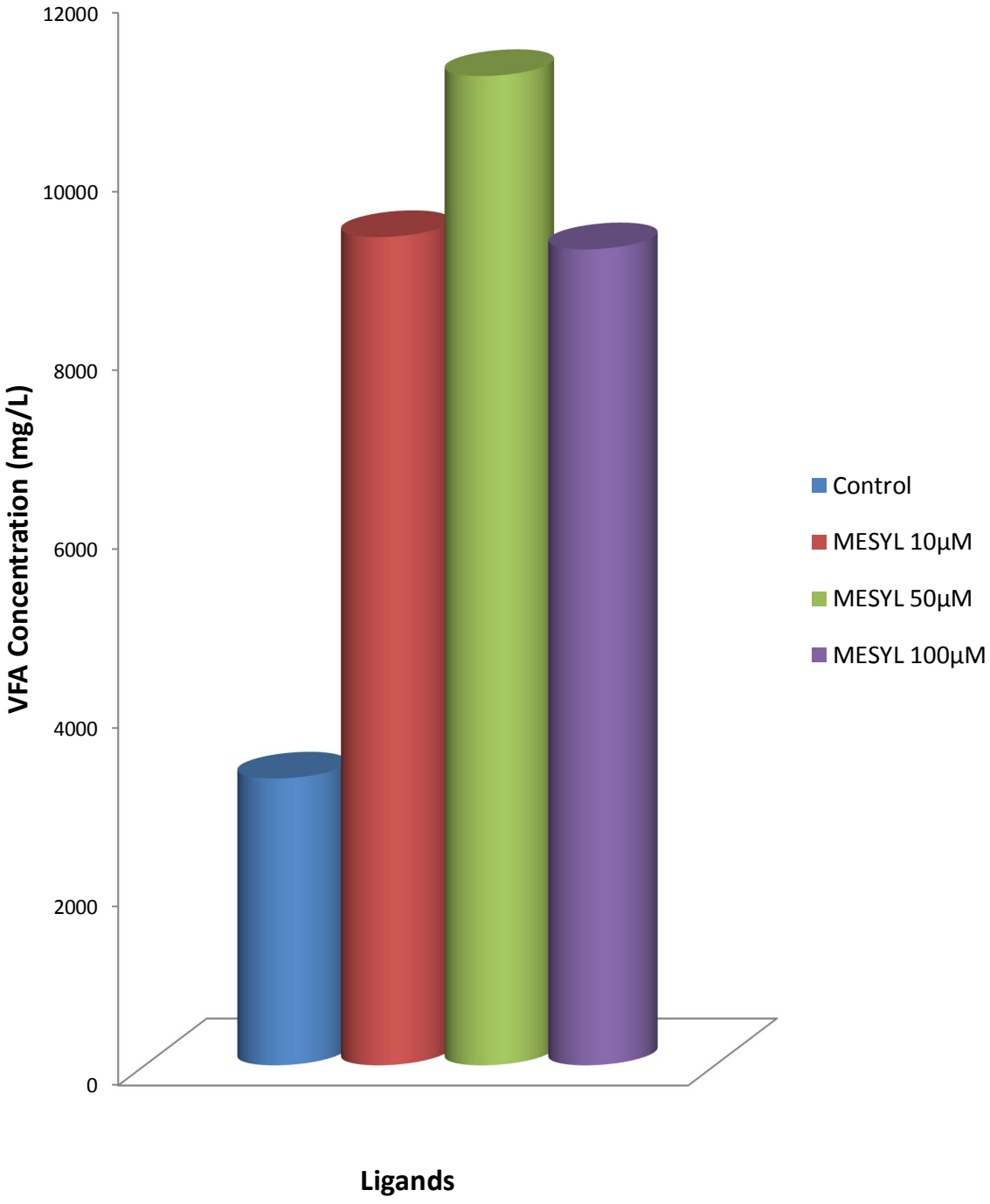


Figure 4.26c: Concentration of VFA in CH MESYL Digester

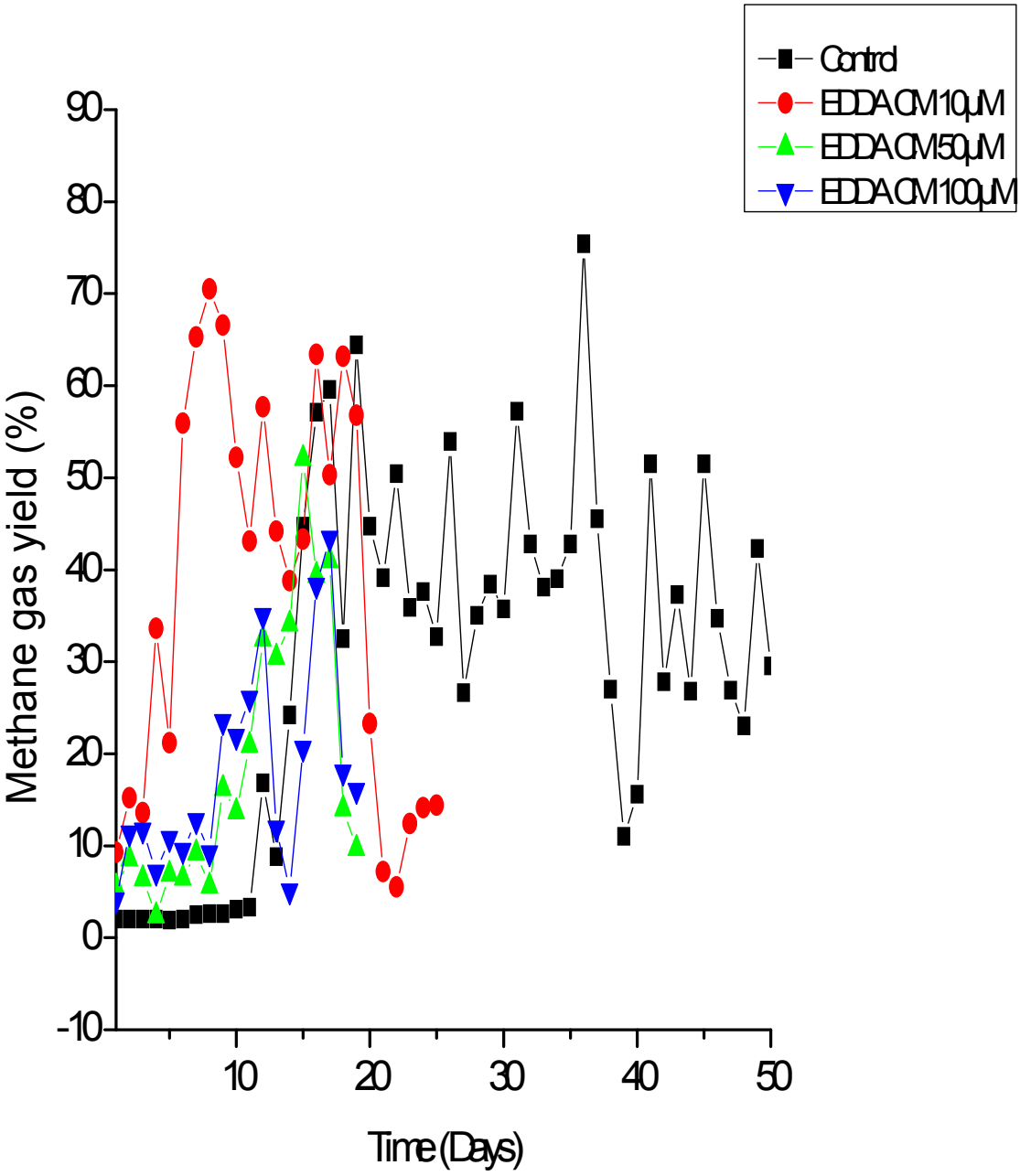


Figure 4.27: Effect of Increasing Chelating Ligand Concentration on HRT in CM EDDA Digester

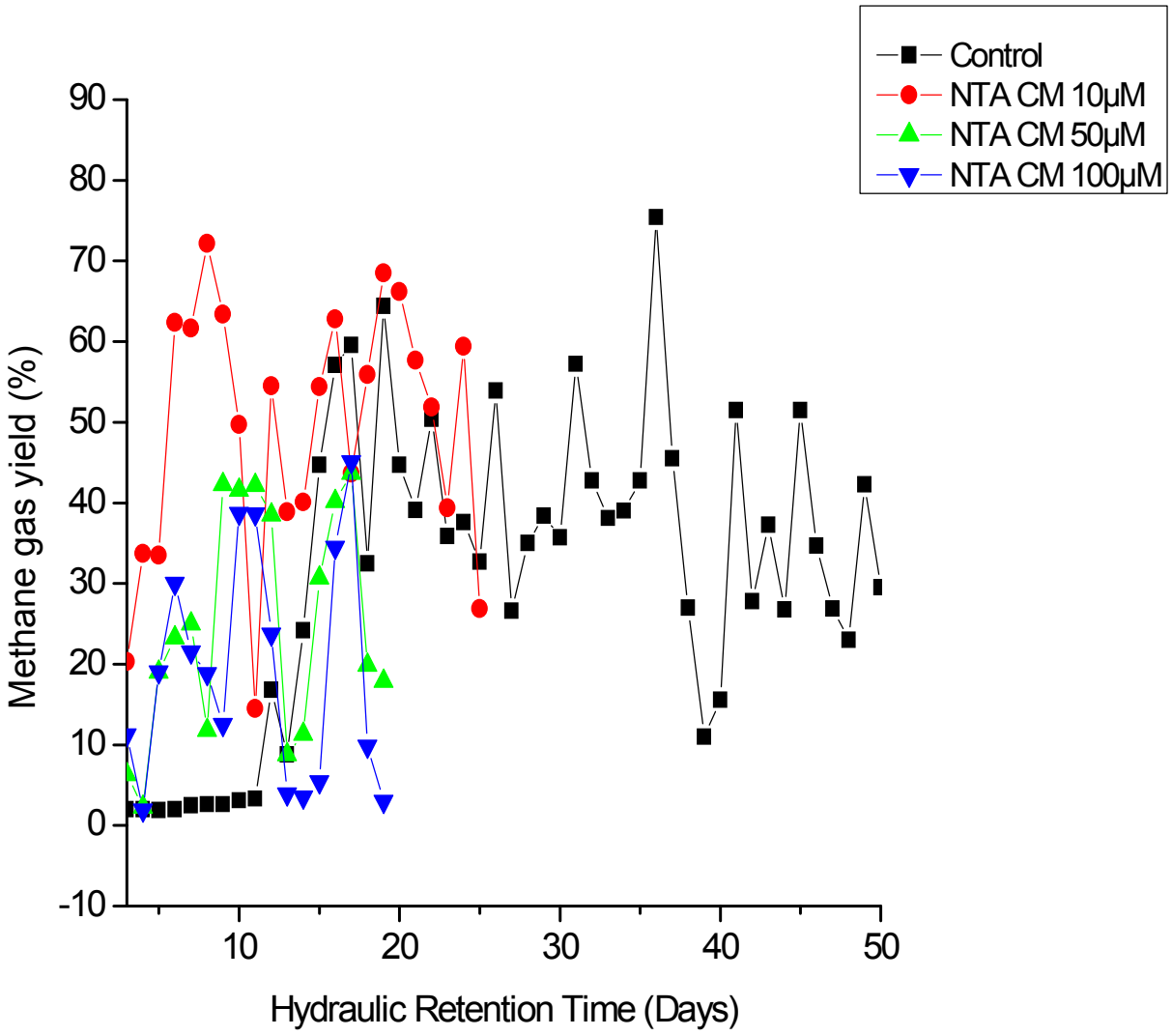


Figure 4.28: Effect of Increasing Chelating Ligand Concentration on HRT in CM NTA Digester

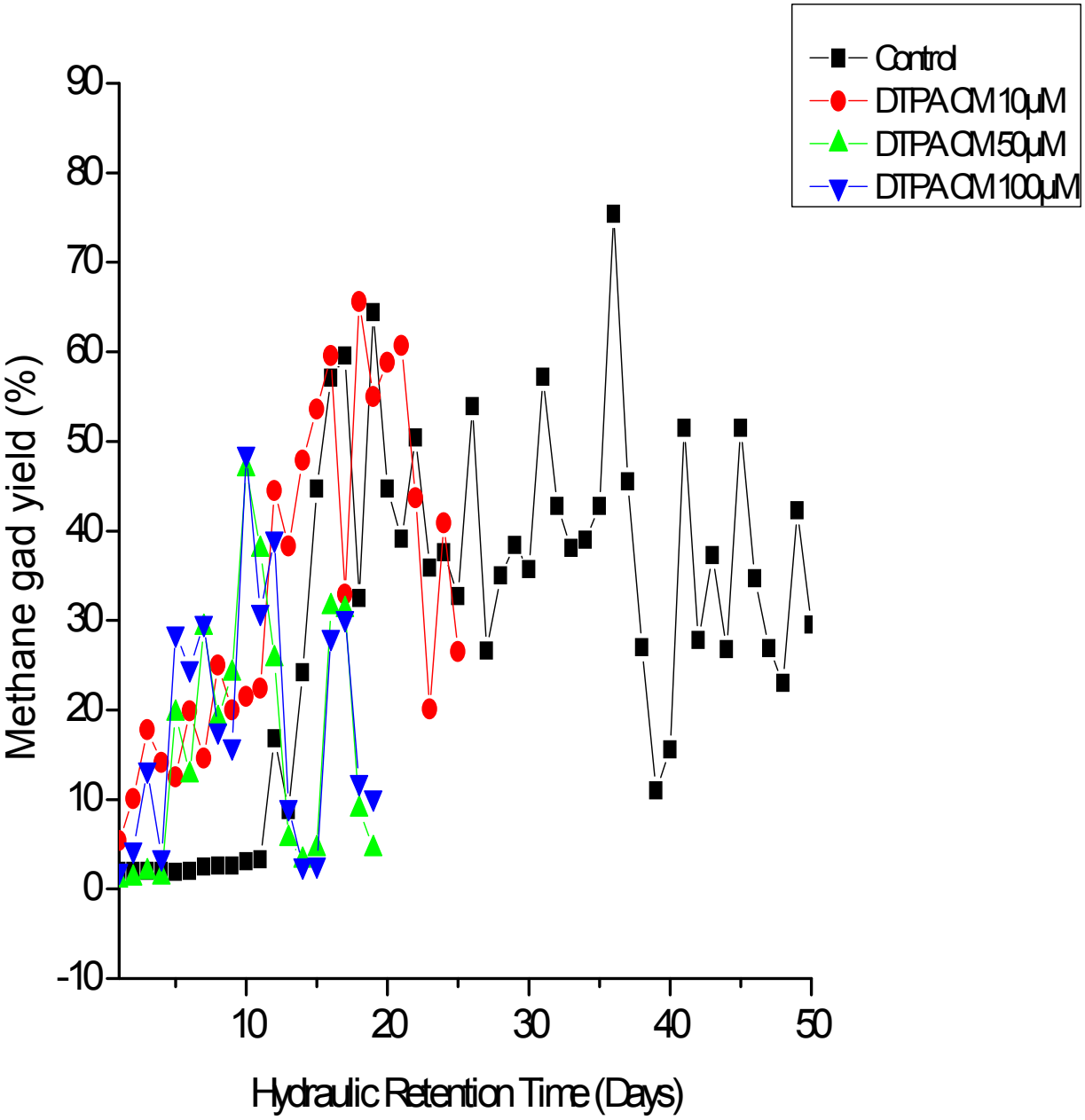


Figure 4.29: Effect of Increasing Chelating Ligand Concentration on HRT in CM DTPA Digester

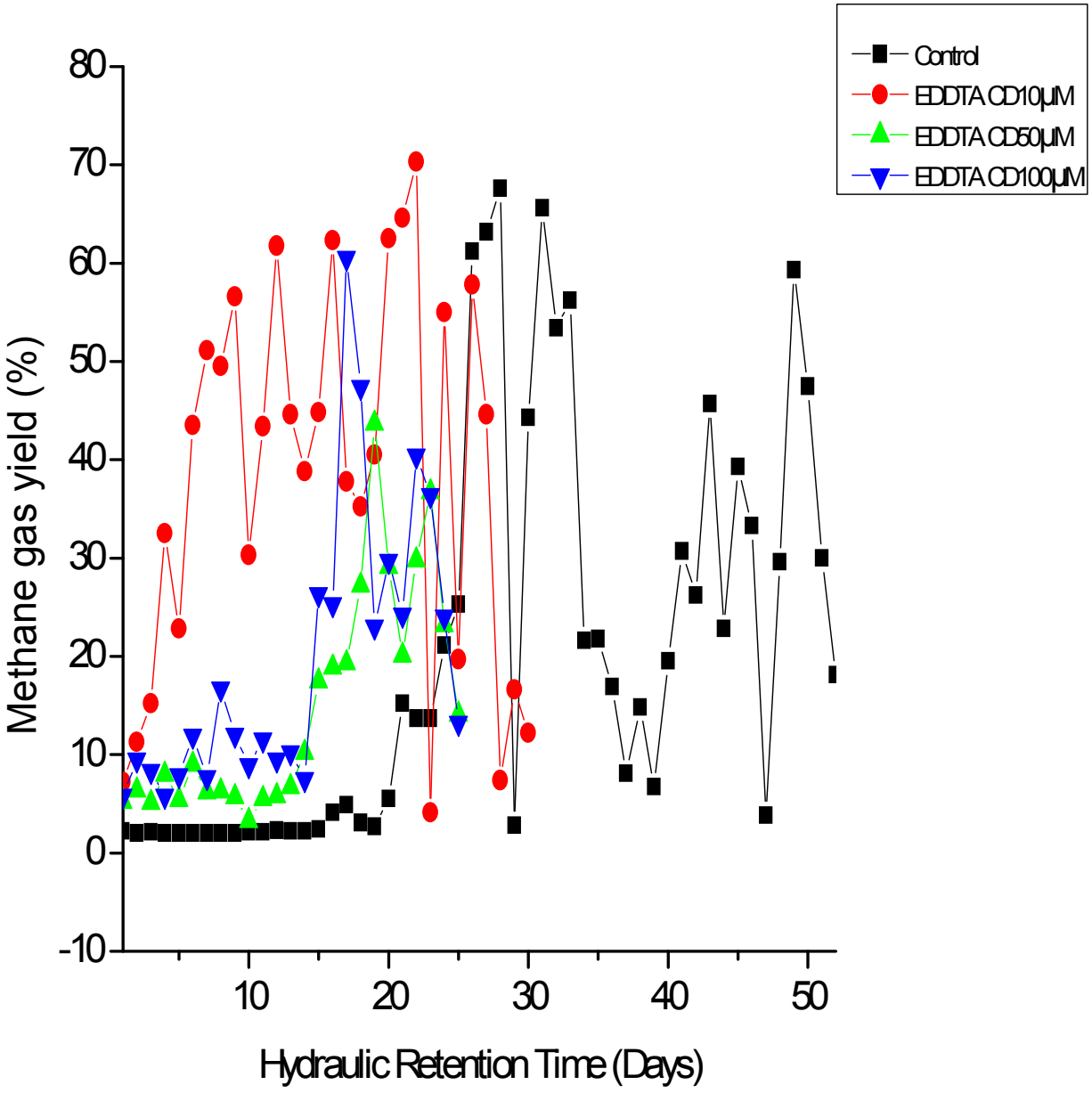


Figure 4.30: Effect of Increasing Chelating Ligand Concentration on HRT in CD EDTA Digester

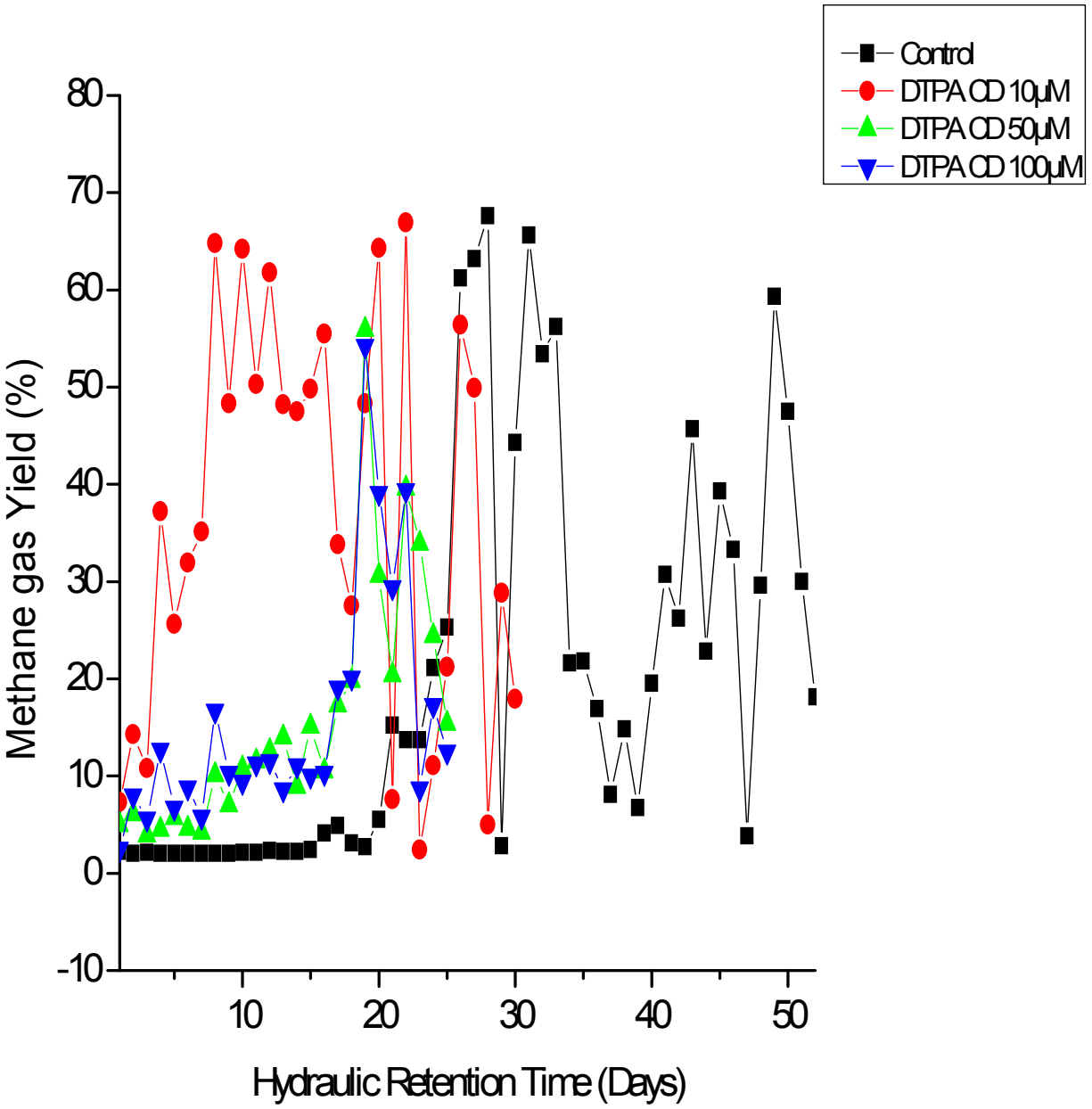


Figure 4.31: Effect of Increasing Chelating Ligand Concentration on HRT in CD DTPA Digester

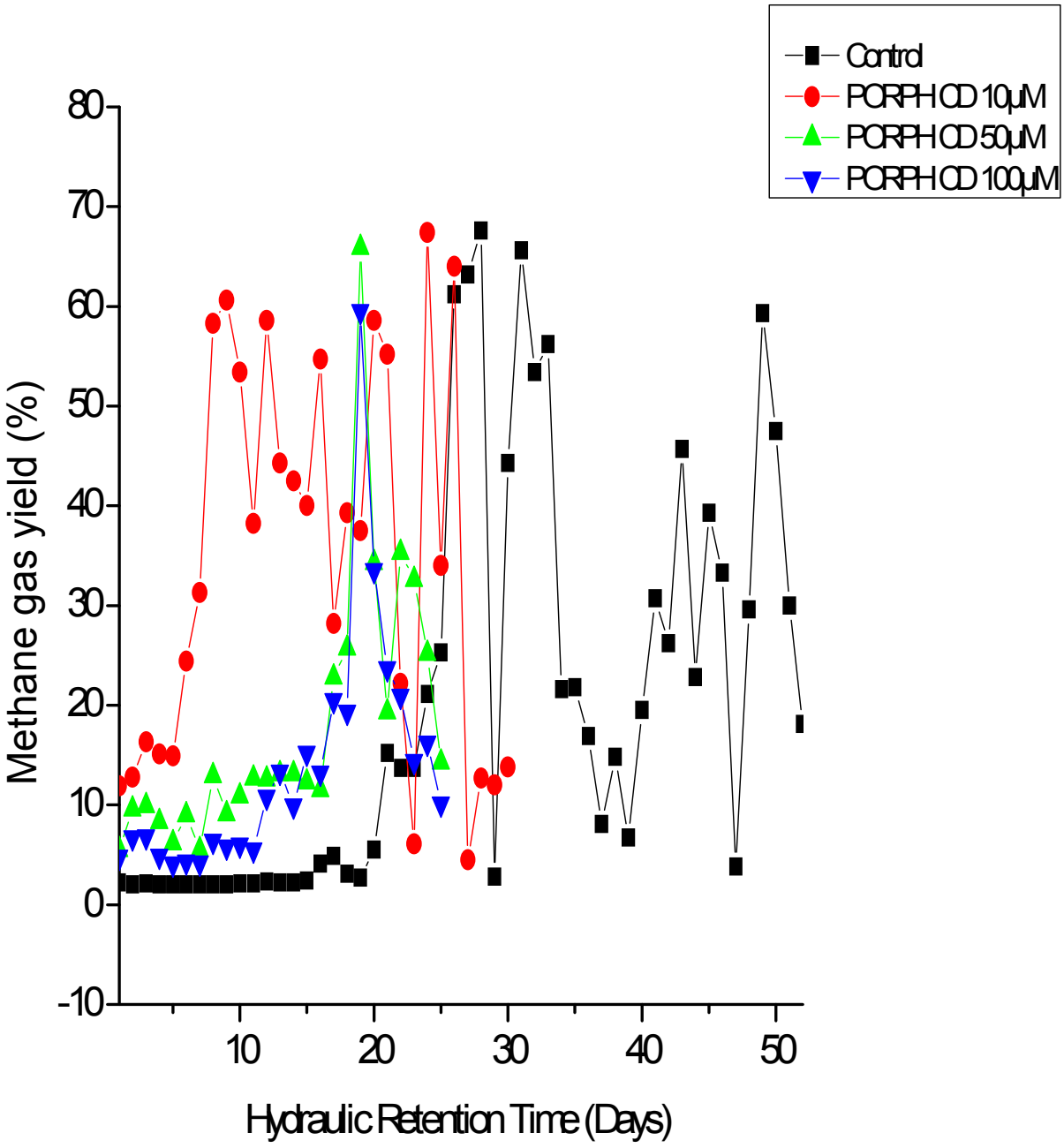


Figure 4.32: Effect of Increasing Chelating Ligand Concentration on HRT in CD PORPH Digester

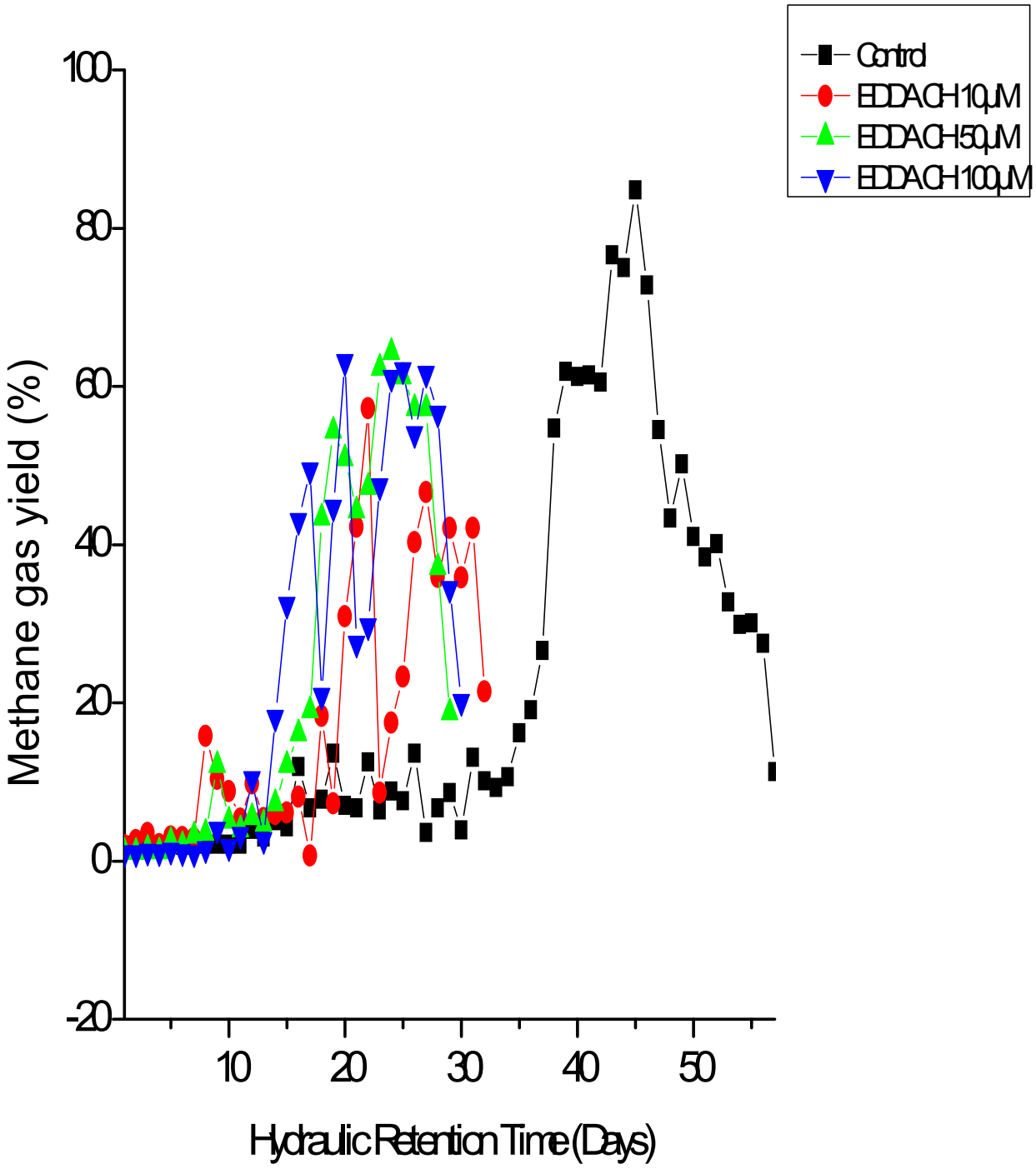


Figure 4.33: Effect of Increasing Chelating Ligand Concentration on HRT in CH EDDA Digester

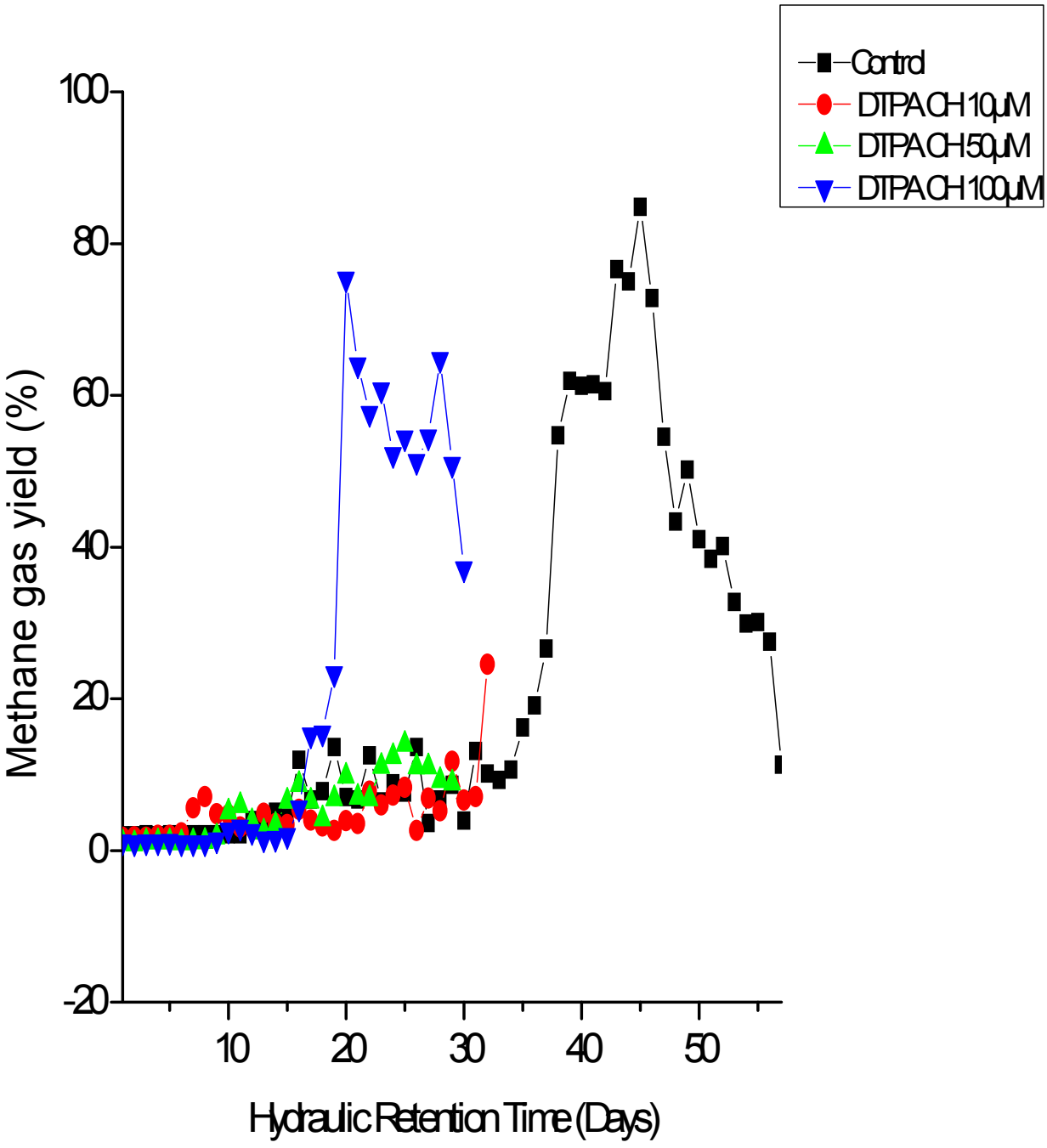


Figure 4.34: Effect of Increasing Chelating Ligand Concentration on HRT in CH DTPA Digester

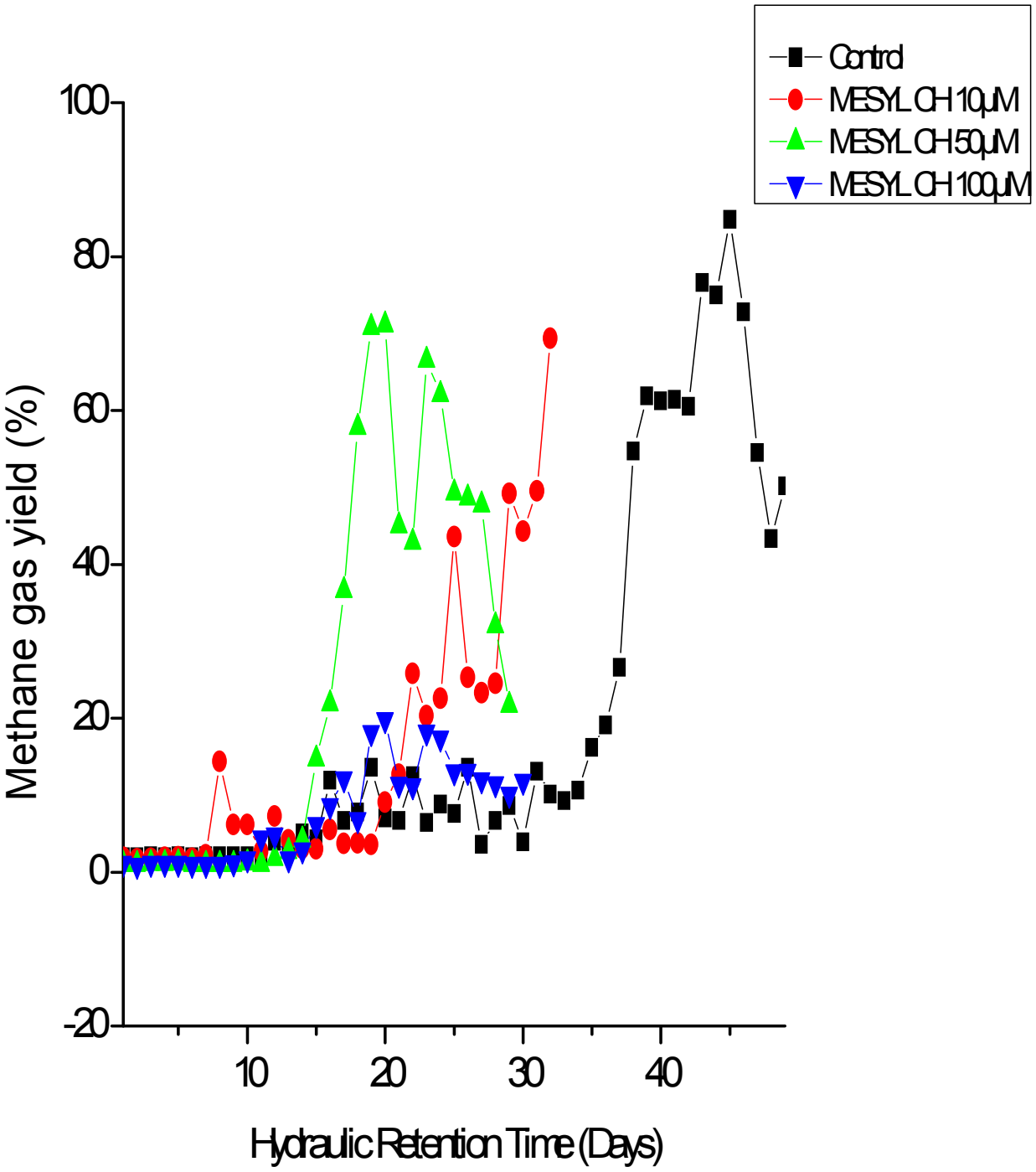


Figure 4.35: Effect of Increasing Chelating Ligand Concentration on HRT in CH MESYL Digester

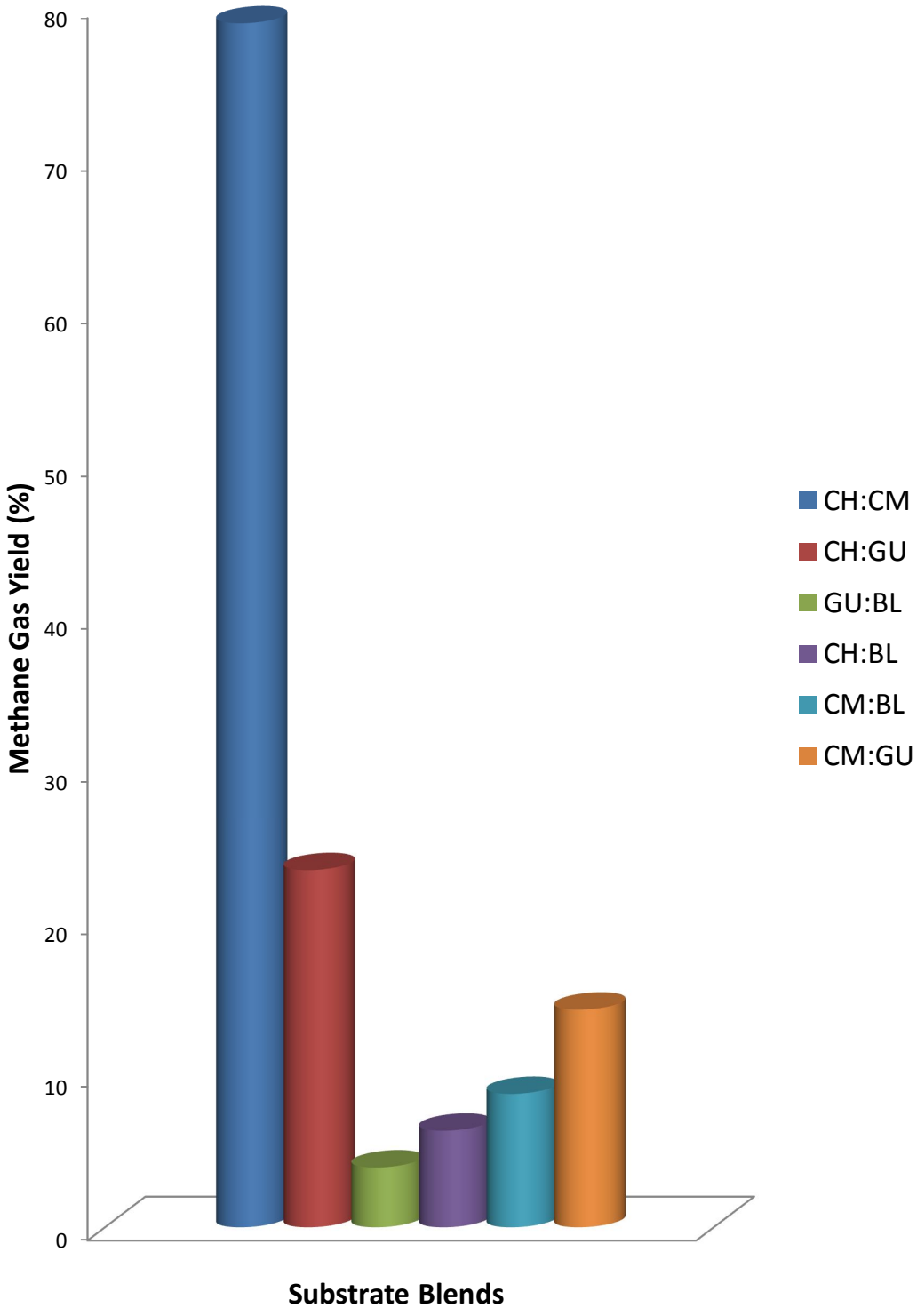


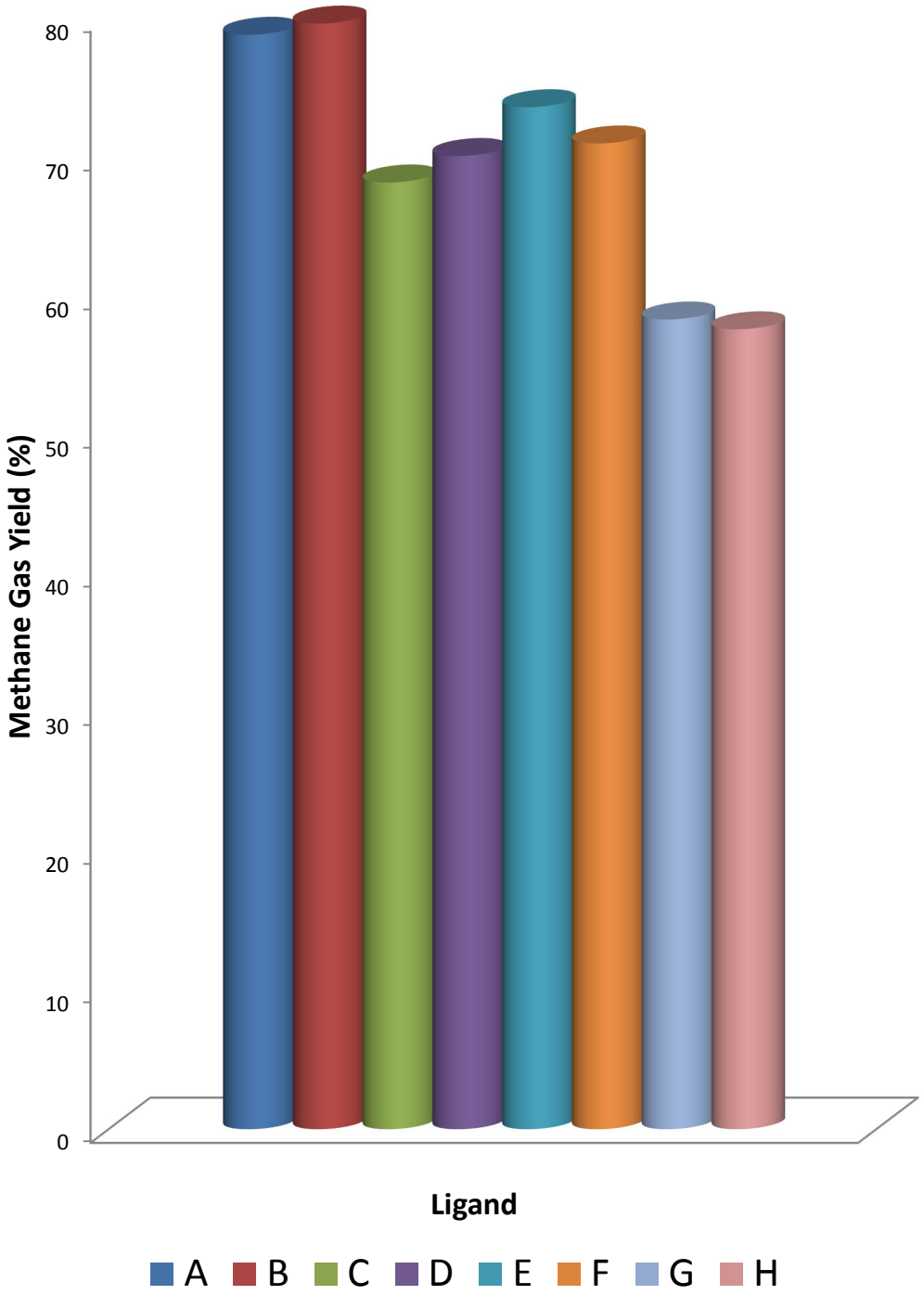
Figure 4.36: Percentage of CH_4 gas produced from the Different Blends.

4.11 Effect of Chelating Ligand on CH_4 gas Yield and VFA concentration in CH: CM Co-digestion system

Studies were carried out on the effect of the addition of seven different chelating ligands namely EDDA, EDTA, DTPA, NTA, PORPH, MESYL, and PENI into the best performing co-digestion system namely CH: CM; the results are presented in Figure 4.37. The Figure 4.37 shows the plot of percentage CH_4 gas yield from the CH: CM Co-digester systems containing chelating ligands, against the seven chelating ligands labelled A – H. Figure 4.38 showed the effect of the addition of chelating ligand on VFA concentration.

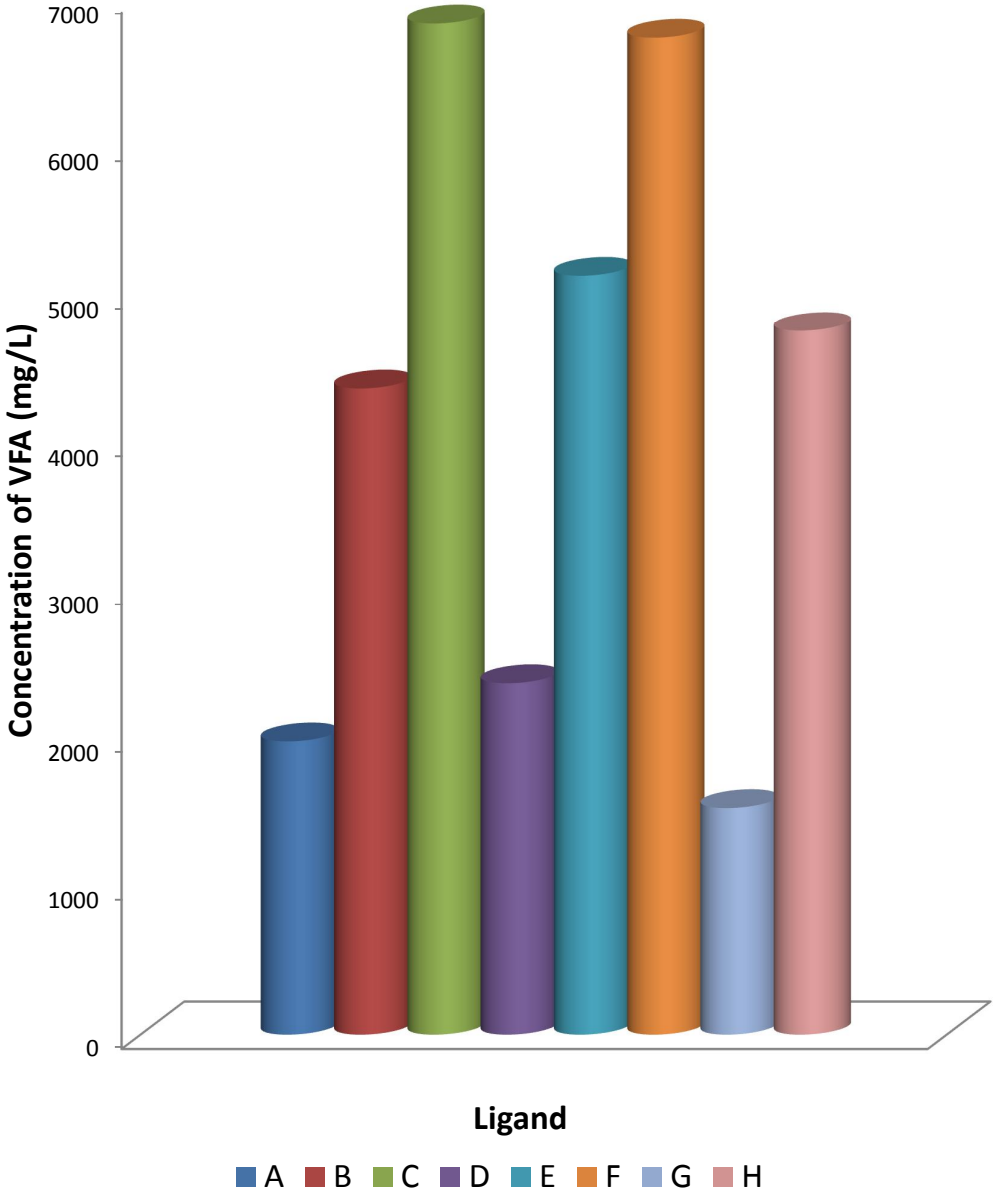
4.12 Effect of Increasing Concentration of Chelating Ligand on CH_4 gas Yield and VFA Concentration in the Co-digestion Systems

From all the chelating ligands introduced into the CH: CM co-digestion systems, EDDA, DTPA and MESYL chelating ligands performed best with highest percentage CH_4 gas yield. The results presented in Figures 4.39 – Figures 4.40 shows the effect of increasing EDDA, DTPA and MESYL chelating ligand concentrations from 10 μM to 100μM, on the CH_4 gas yield and VFA concentrations within the CH: CM co-digestion system. Figure 4.39 was obtained by plotting the percentage yield of CH_4 gas against the various increasing chelating ligand concentrations. While Figures 4.40 was obtained by plotting the graph of VFA concentration on mg/L against the increasing chelating ligand concentrations within the EDDA, DTPA and MESYL CH: CM co-digestion systems.



Control**(A)**, EDDA **(B)**, NTA **(C)**, EDTA **(D)**, DTPA **(E),** MESYL **(F),** PORPH **(G)**, PENI **(H)**

Figure 4.37: Effect of Addition of Chelating Ligands on Co-digestion CH:CM



Control **(A)**, EDDA**(B)**, NTA**(C)**, EDTA**(D)**, DTPA**(E)**, MESYL**(F)**, PORPH**(G)**, PENI**(H)**

Figure 4.38: Effect of Chelating Ligands on VFA Concentration in Co-digestion CH:CM

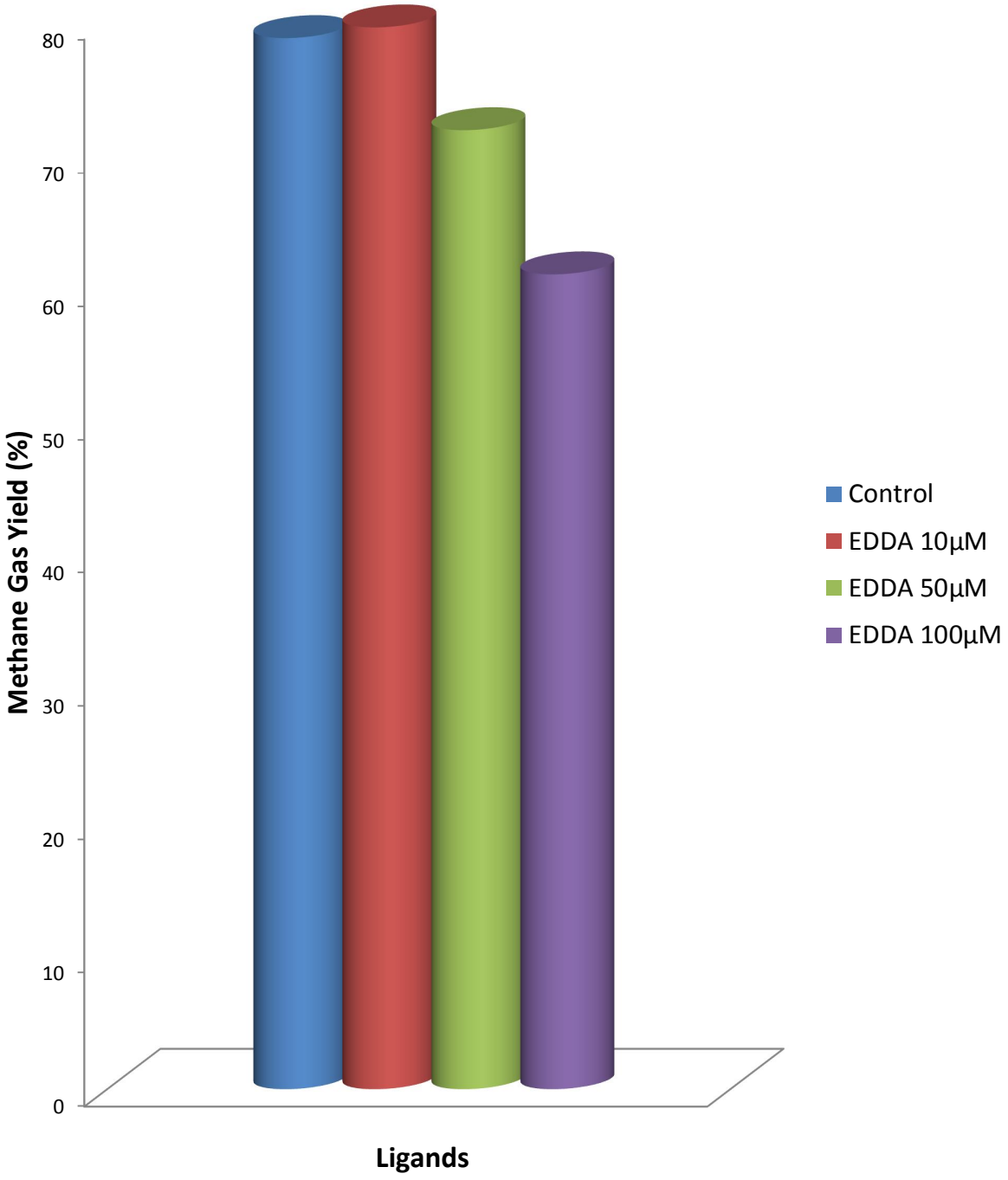


Figure 4.39a: Effect of Increasing EDDA CH: CM Concentration on CH_4 Yield

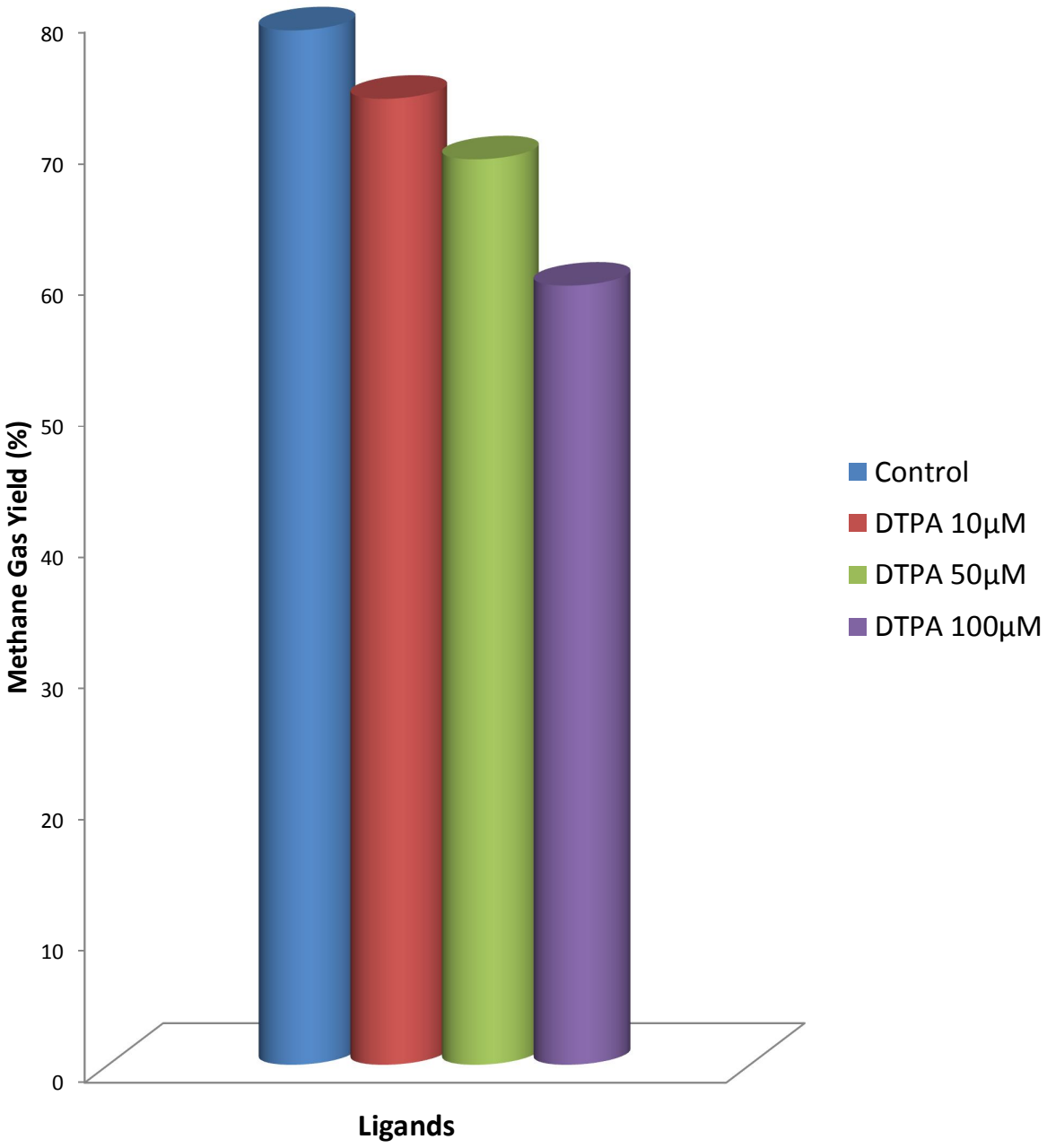


Figure 4.39b: Effect of Increasing DTPA CH: CM Concentration on CH_4 Yield

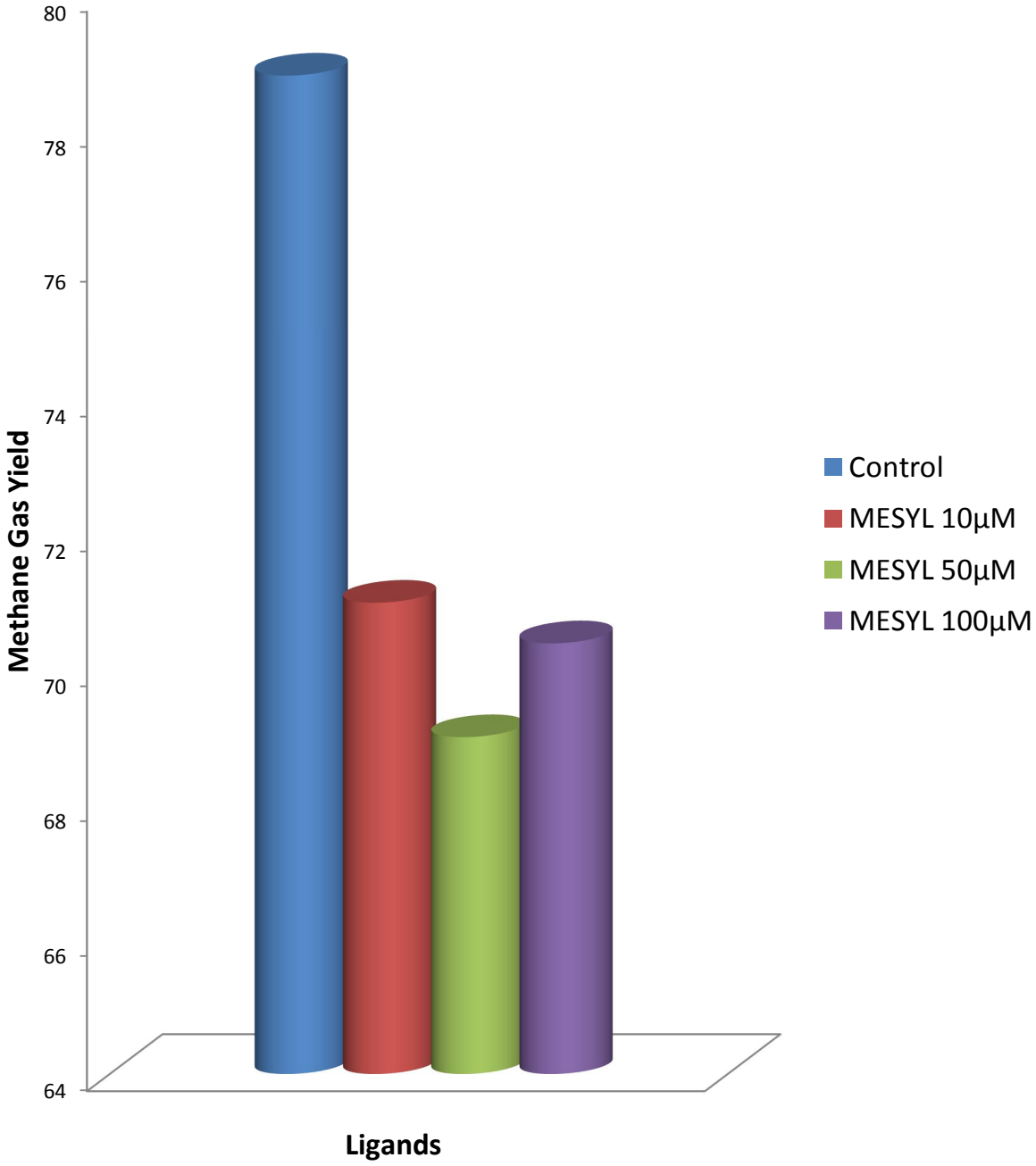


Figure 4.39c: Effect of Increasing MESYL CH: CM Concentration on CH_4 Yield

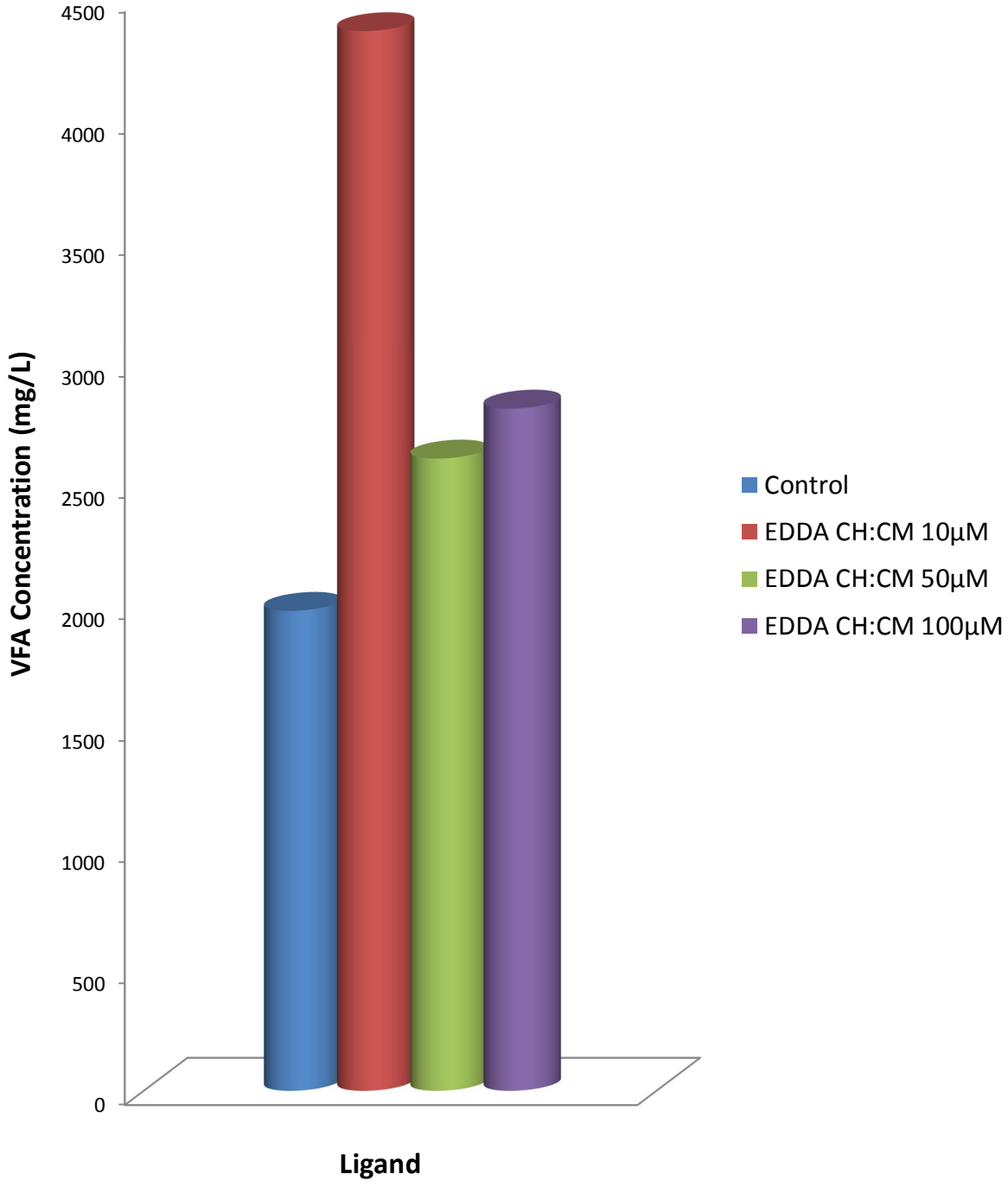


Figure 4.40a: Effect of Increasing EDDA CH: CM Concentration on VFA

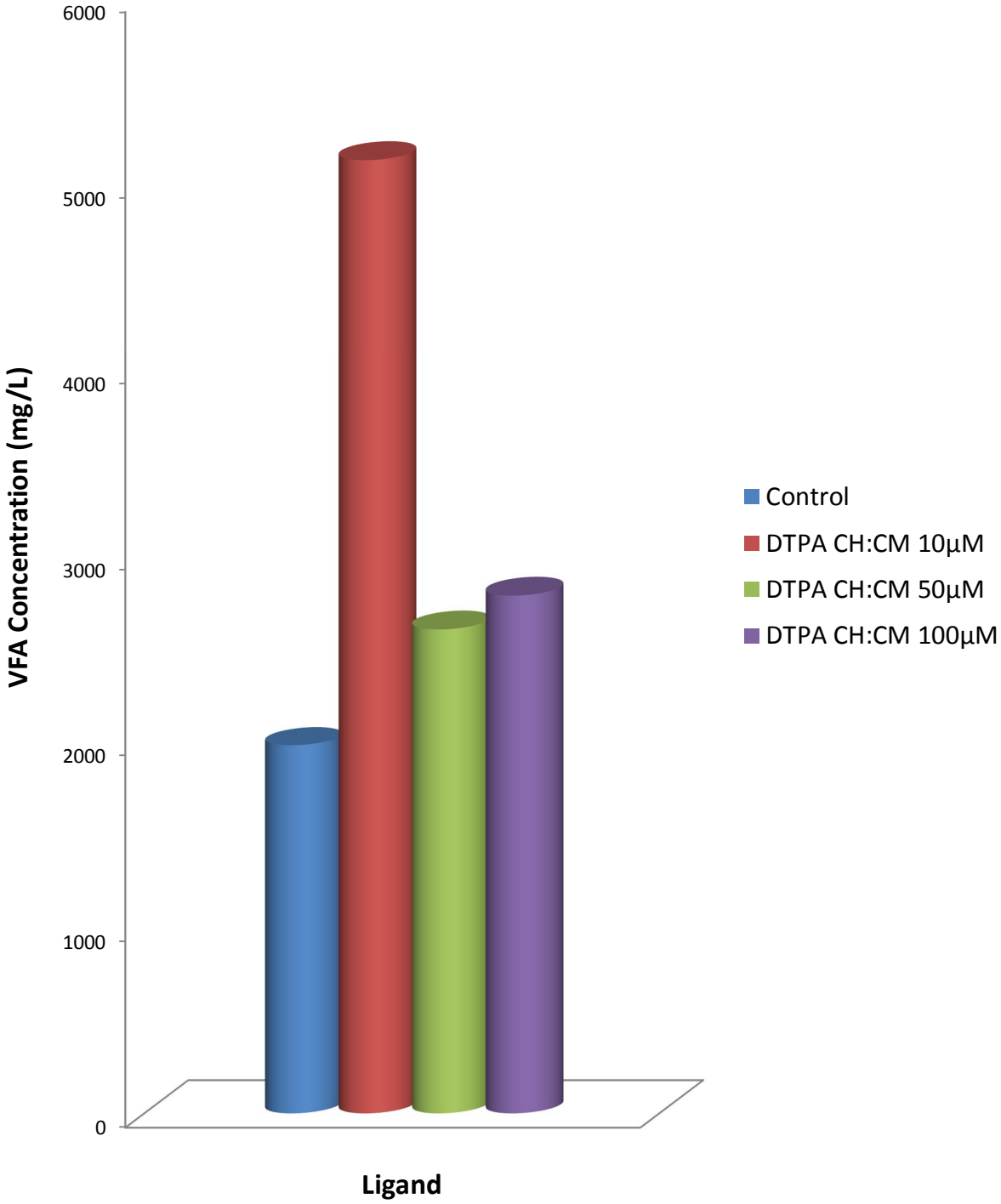


Figure 4.40b: Effect of Increasing DTPA CH: CM Concentration on VFA

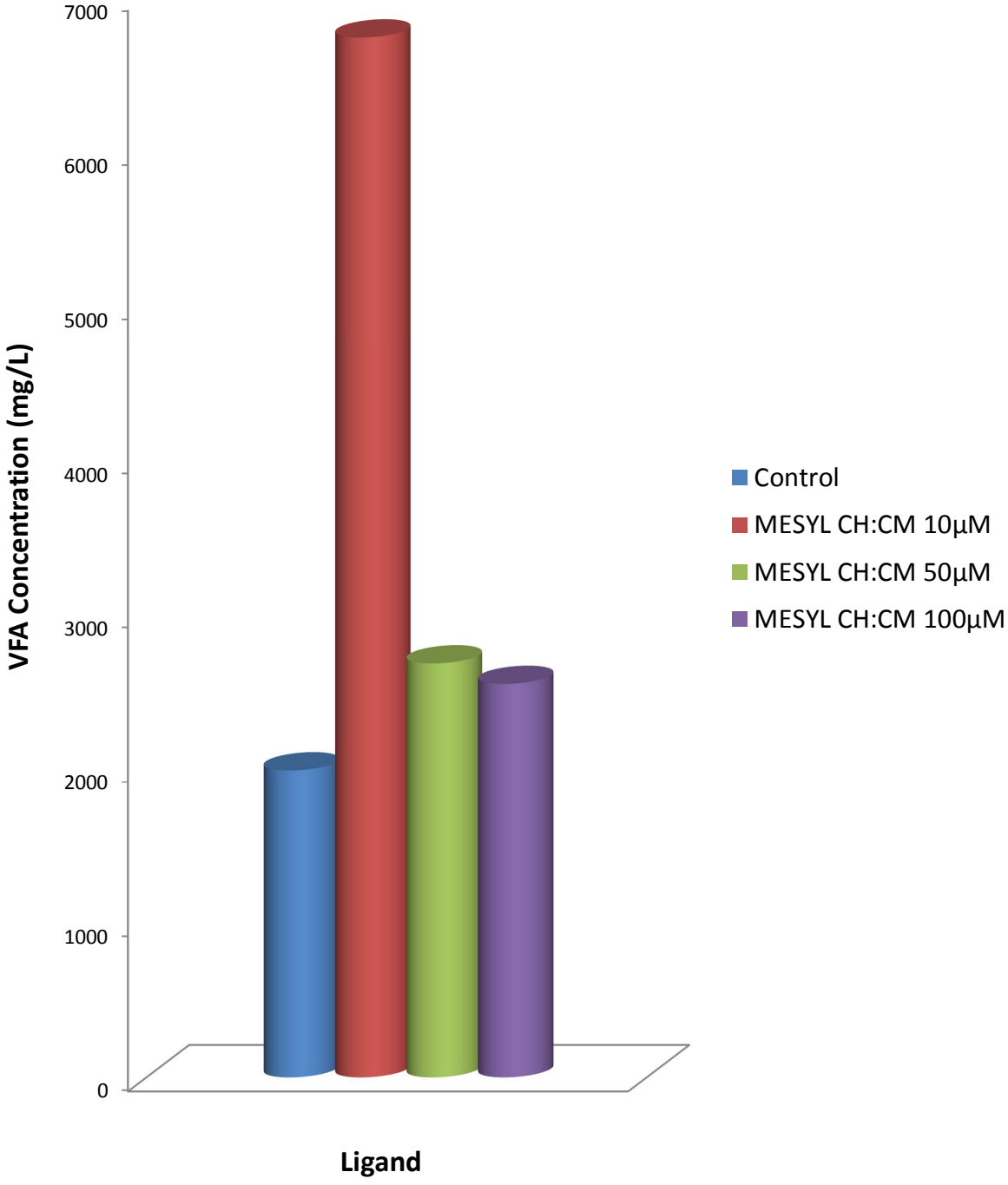


Figure 4.40c: Effect of Increasing MESYL CH: CM Concentration on VFA

4.13 Effect of Addition of Chelating Ligand on HRT in Co-digestion System

A study on the effect of introduction of chelating ligands to the CH: CM Co-digester on HRT was equally carried out on all the seven chelating ligands (EDDA, NTA, EDTA, DTPA, PORPH, MESYL and PENI) and the best performing chelating ligand systems i.e. EDDA, DTPA and MESYL. Figure 4.41 shows a plot of percentage CH_4 gas yield from the CH; CM Co-digester, containing the seven chelating ligands against time expressed in days. Figures 4.42- 4.44 shows the plot of percentage CH_4 gas yield at increasing chelating ligand concentration, (from a concentration of 10 μM to 100 μM) against Time expressed in days.

4.14 Effect of Addition of Chelating Ligand on H_2S gas produced within the Co-digester system

Increasing concentrations of chelating ligand EDDA, DTPA, MESYL were added to the best performing co-digester CH: CM and the results presented in Figure 4.45. The graph in Figure 4.45 was obtained by plotting concentrations of H_2S gas produced against increasing chelating ligand concentration, from 10 μM to 100 μM.

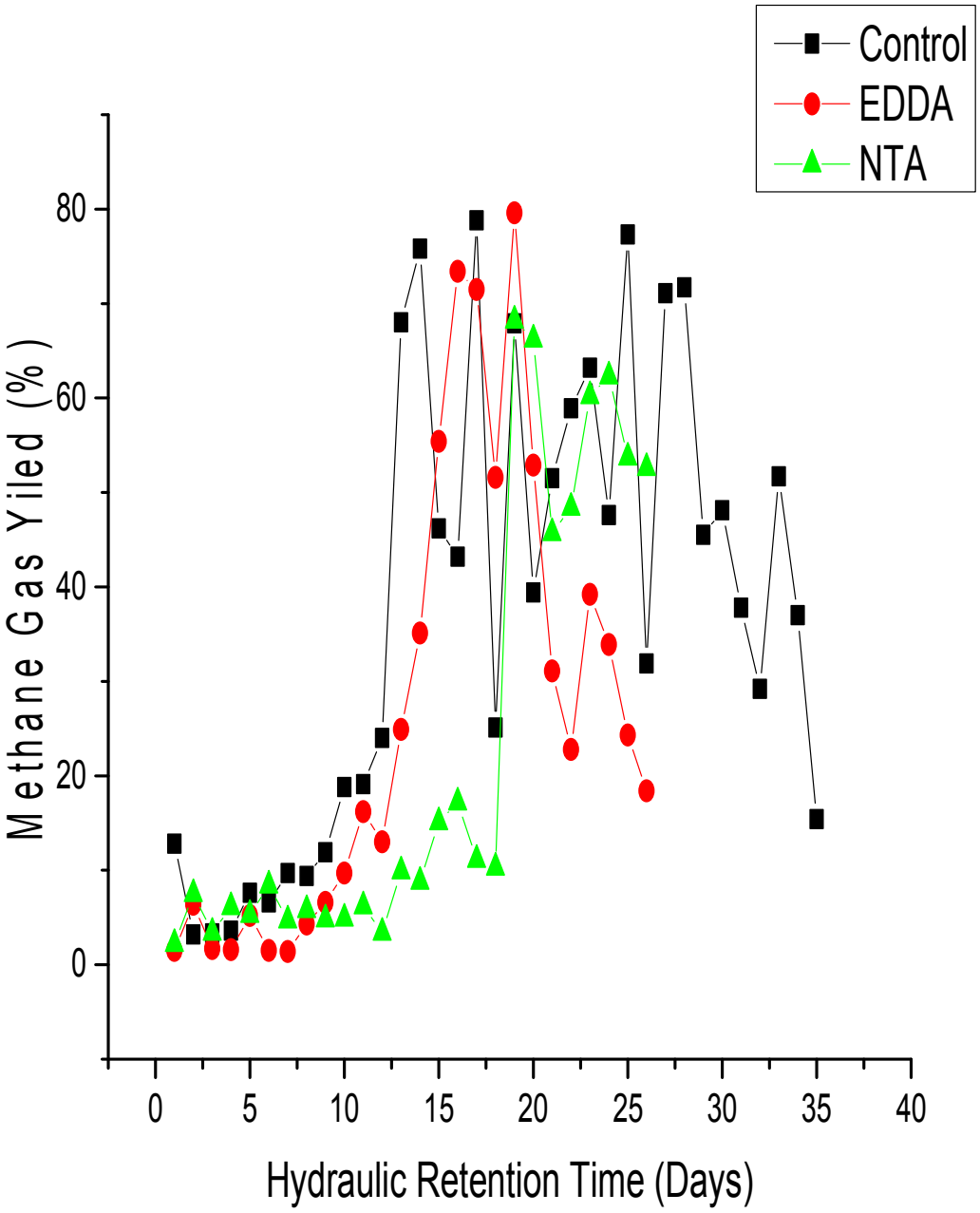


Figure 4.41a: Effect of Addition of Chelating Ligand on HRT for Co-digestion in EDDA and NTA

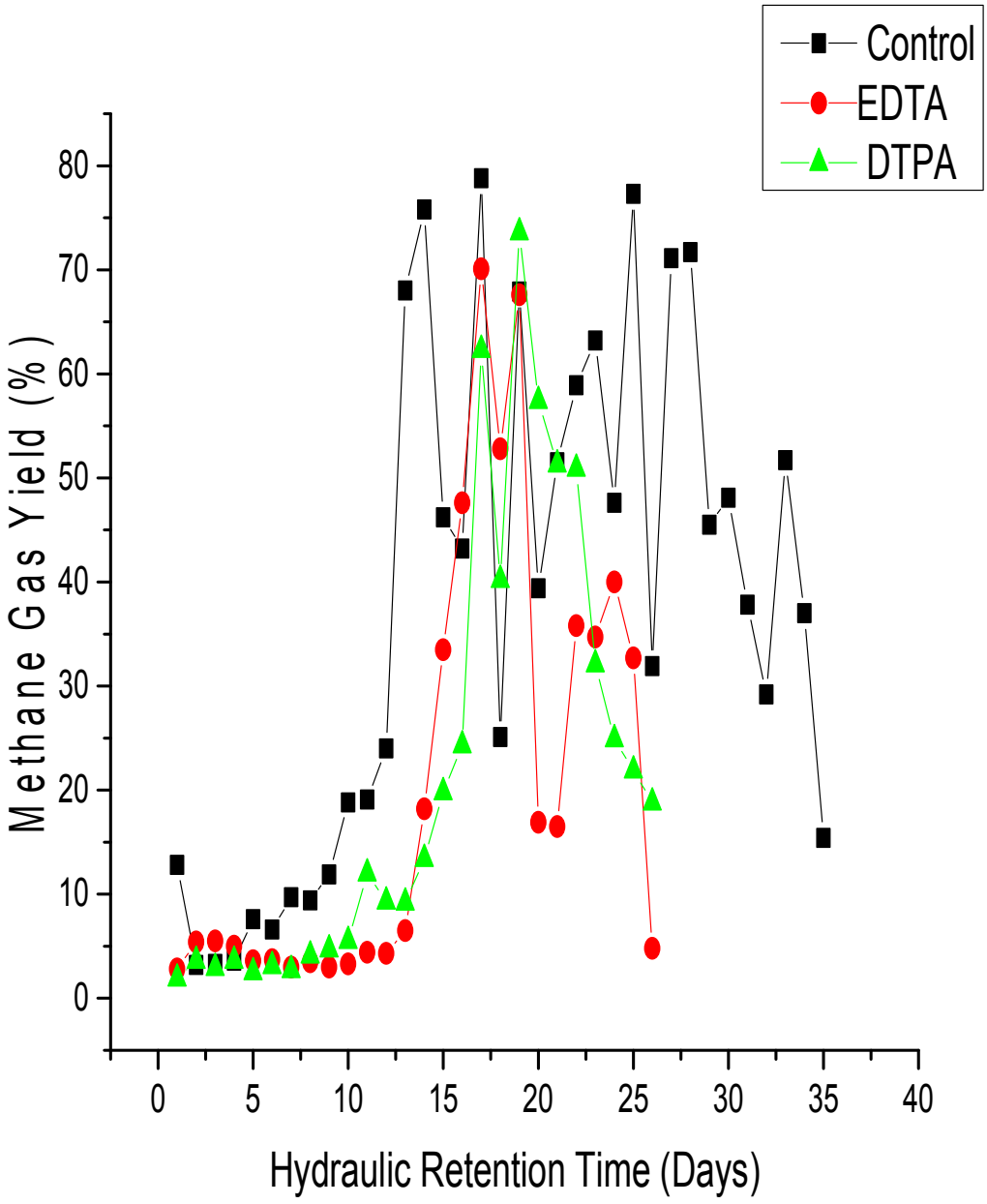


Figure 4.41b: Effect of Addition of Chelating Ligand on HRT for Co-digestion in EDTA and DTPA

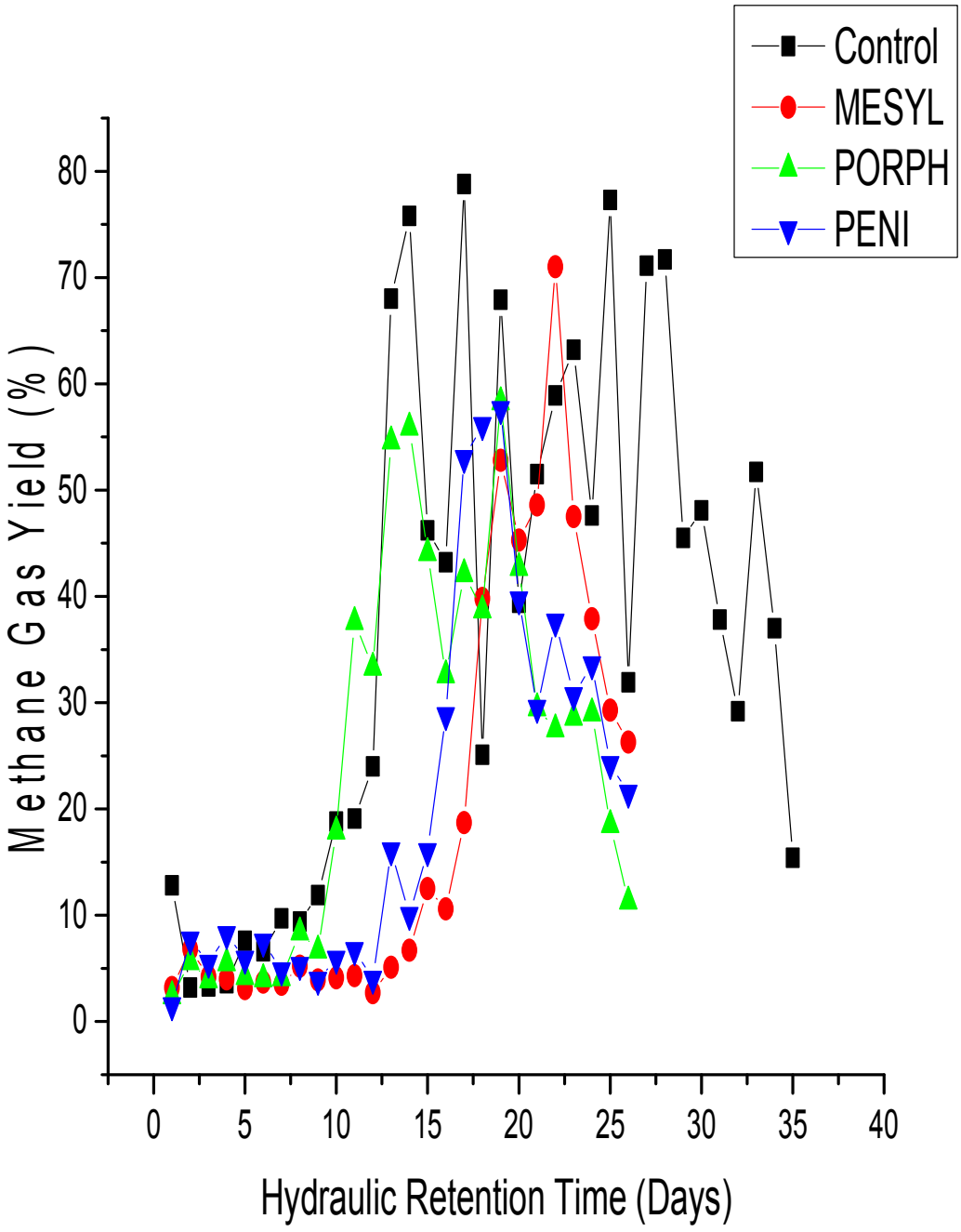


Figure 4.41c: Effect of Addition of Chelating Ligand on HRT for Co-digestion in MESYL, PORPH and PENI

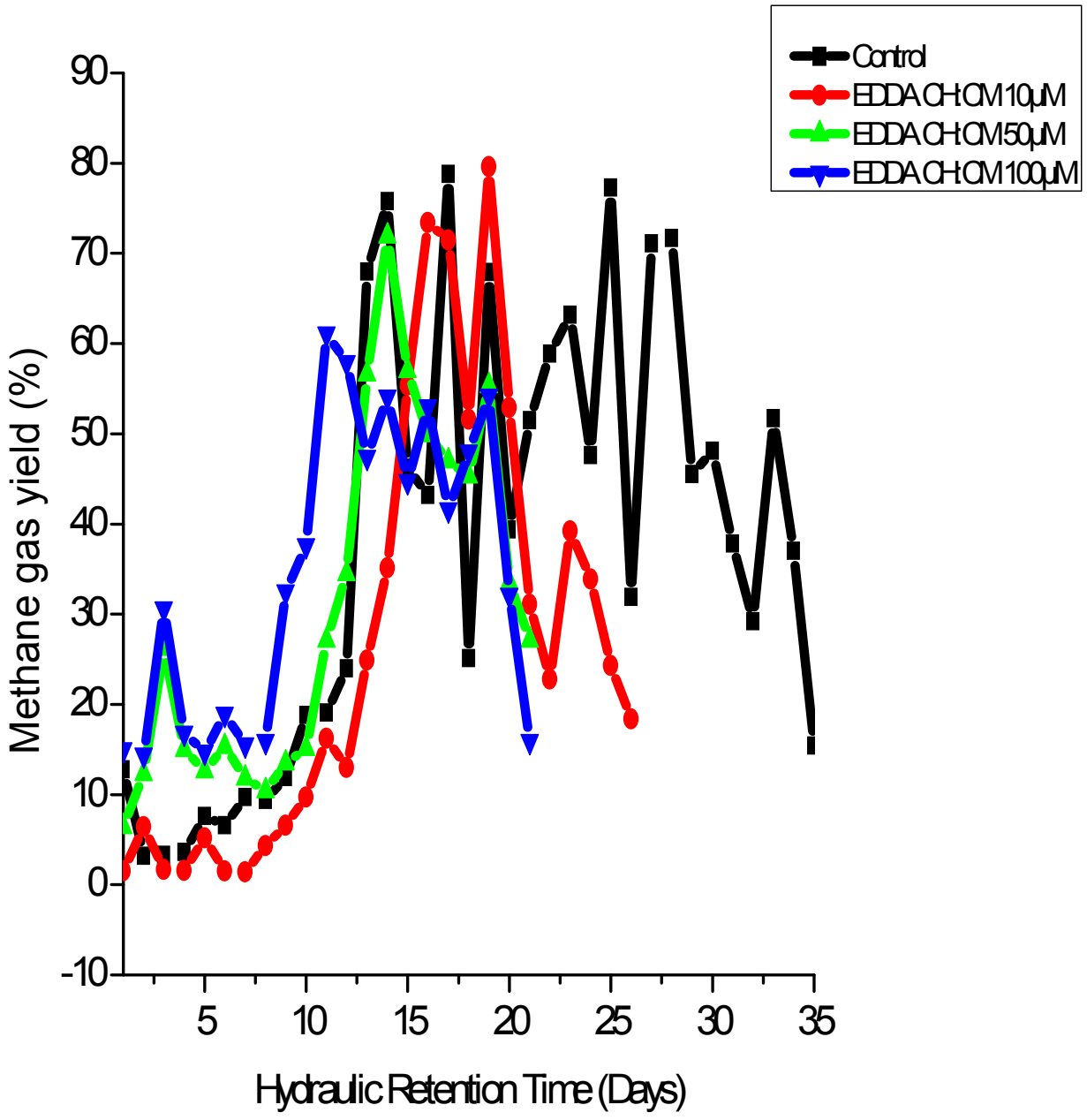


Figure 4.42: Effect of Increasing Concentration of Chelating Ligand on HRT in Co-digestion System containing EDDA

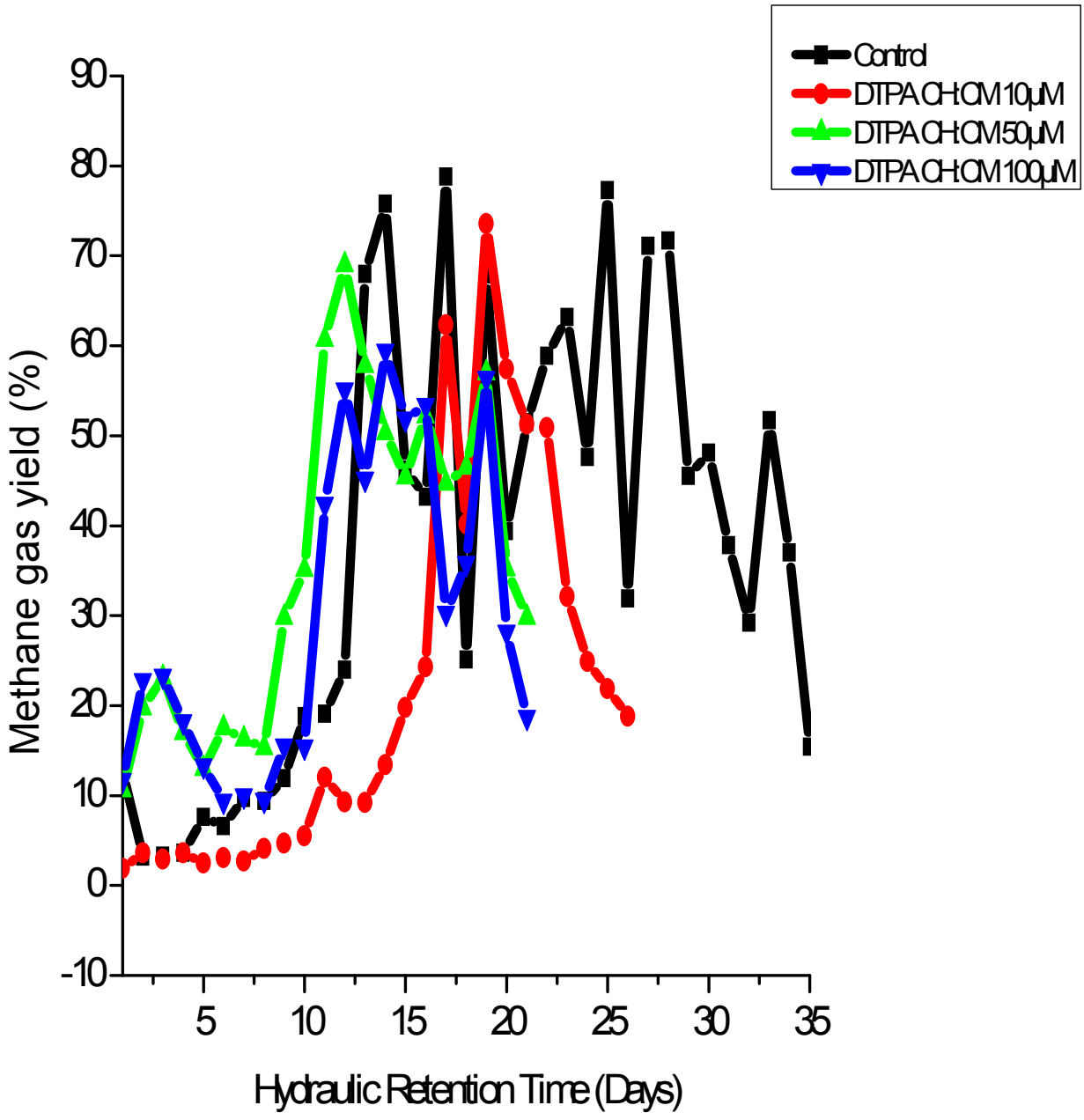


Figure 4.43: Effect of Increasing Concentration of Chelating Ligand on HRT in Co-digestion System containing DTPA

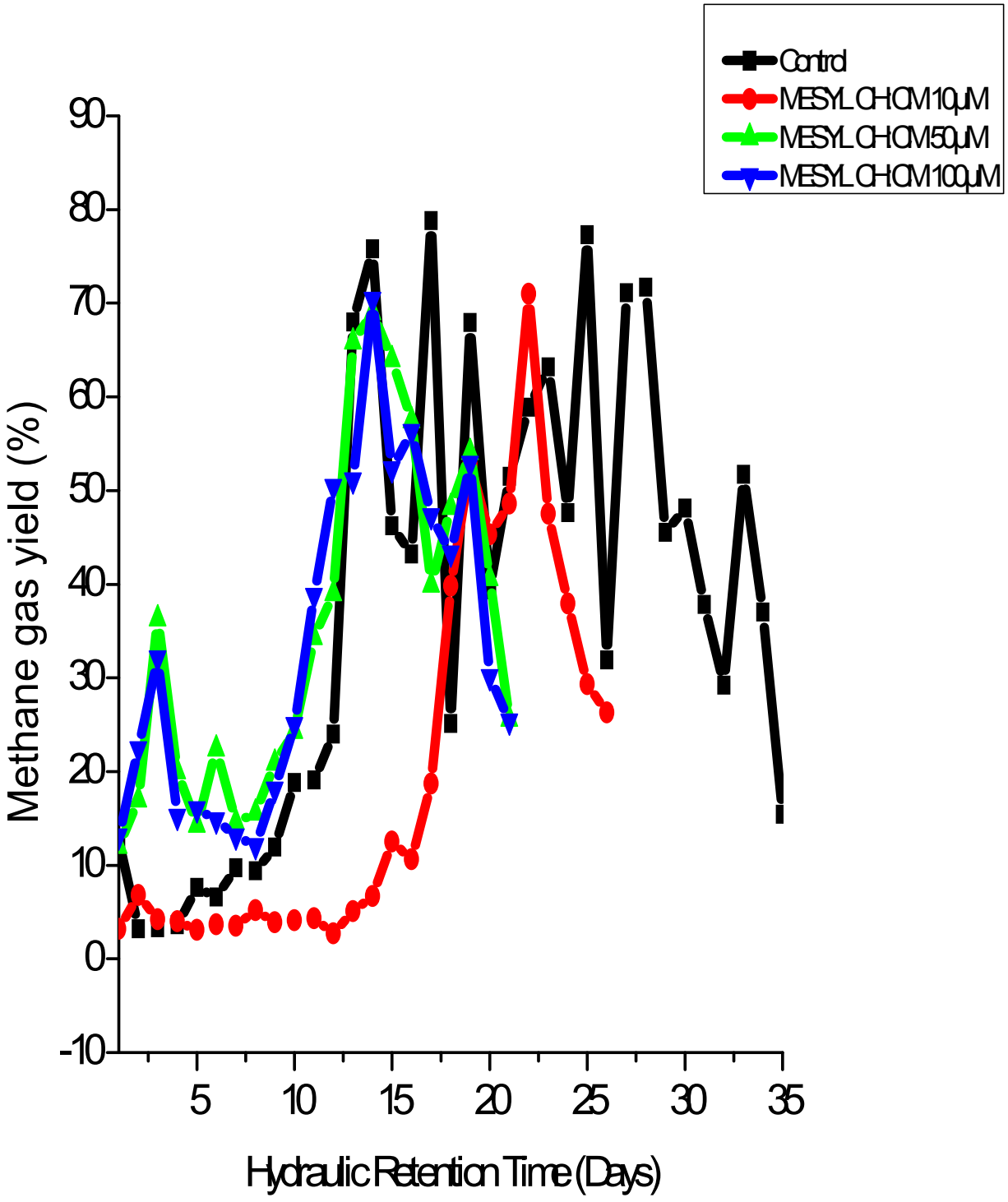


Figure 4.44: Effect of Increasing Concentration of Chelating Ligand on HRT in Co-digestion System containing MESYL

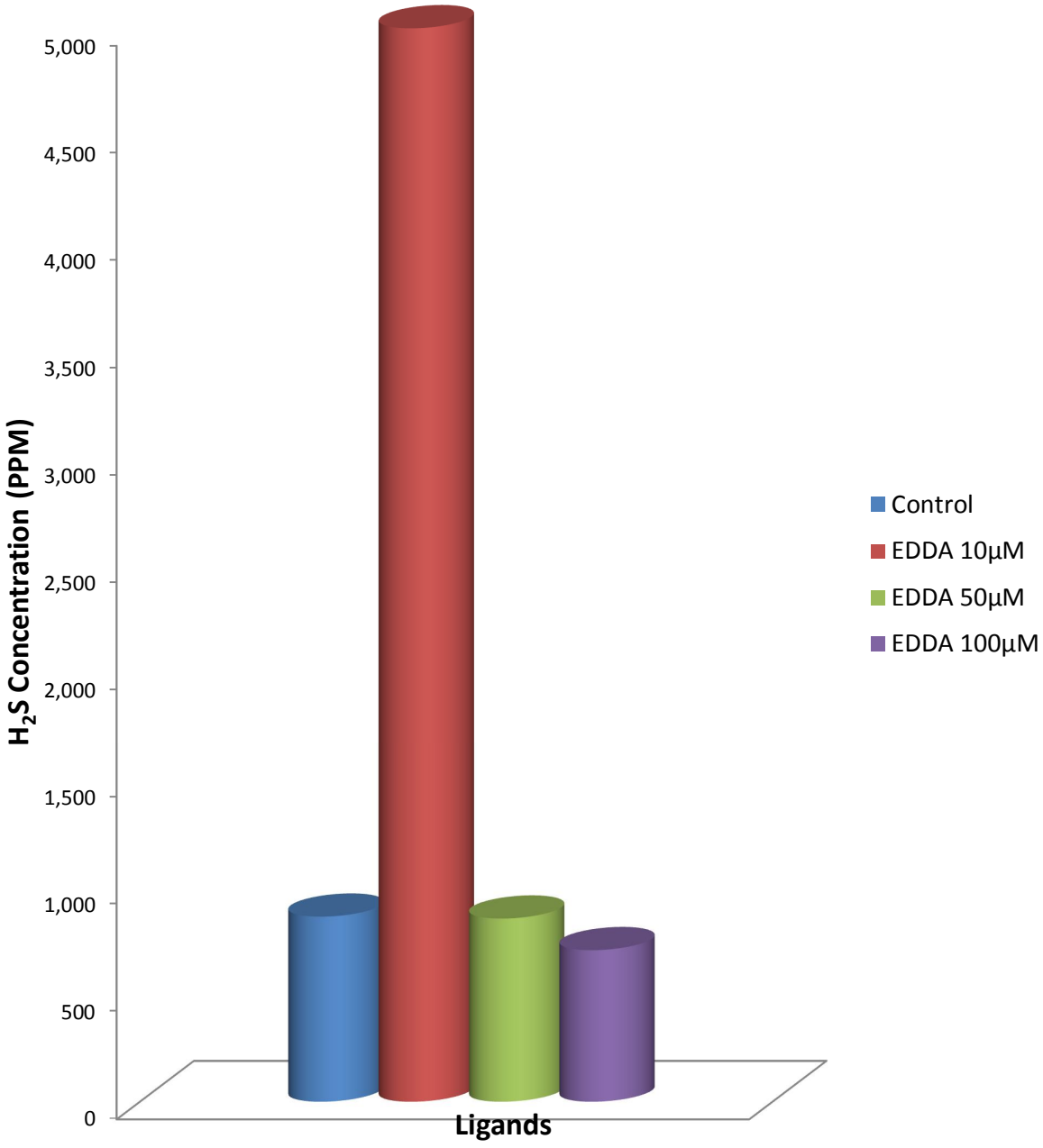


Figure 4.45a: Effect of Increasing EDDA CH: CM concentration on H_2S Concentration

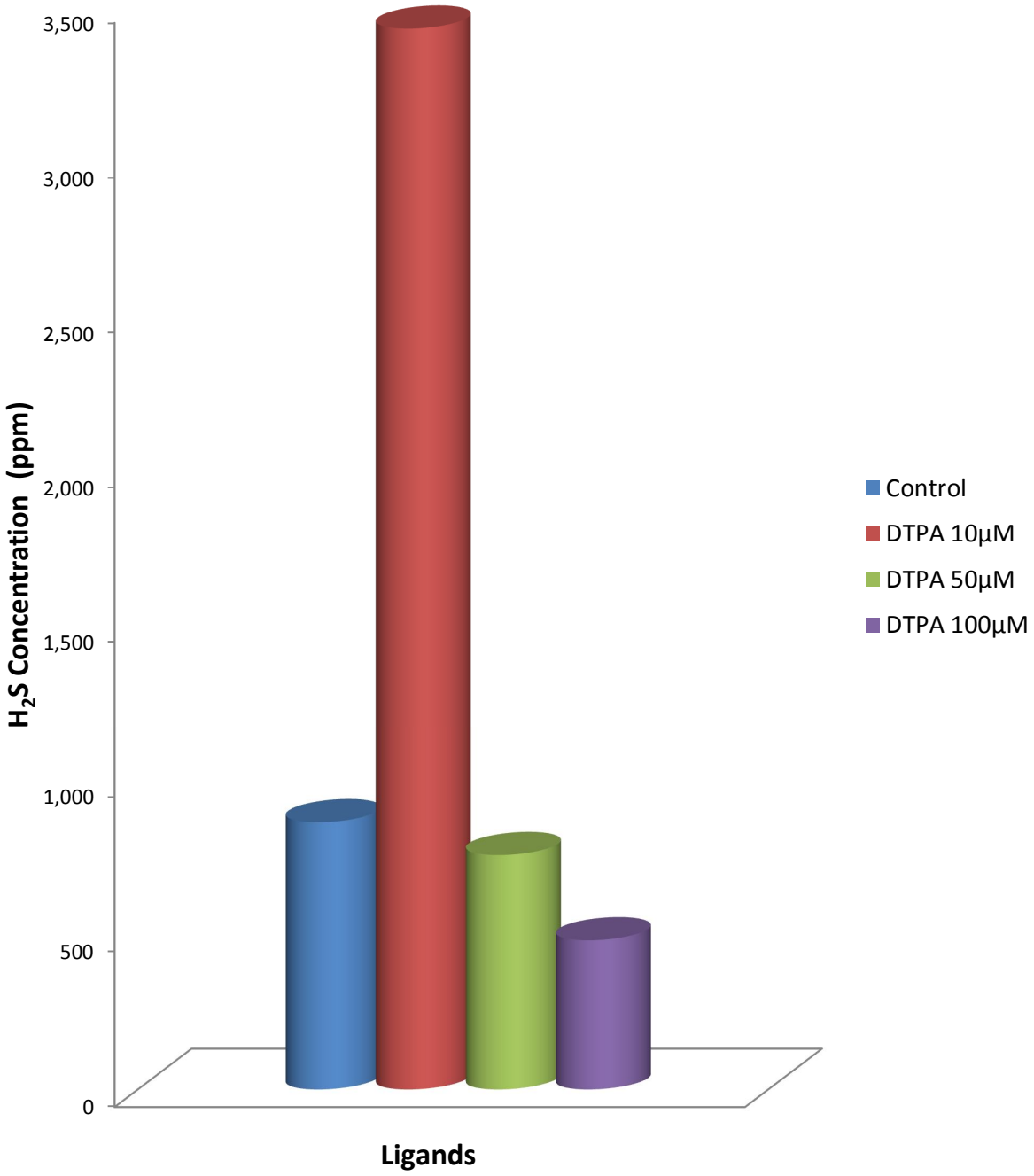


Figure 4.45b: Effect of Increasing DTPA CH:CM concentration on H_2S Concentration

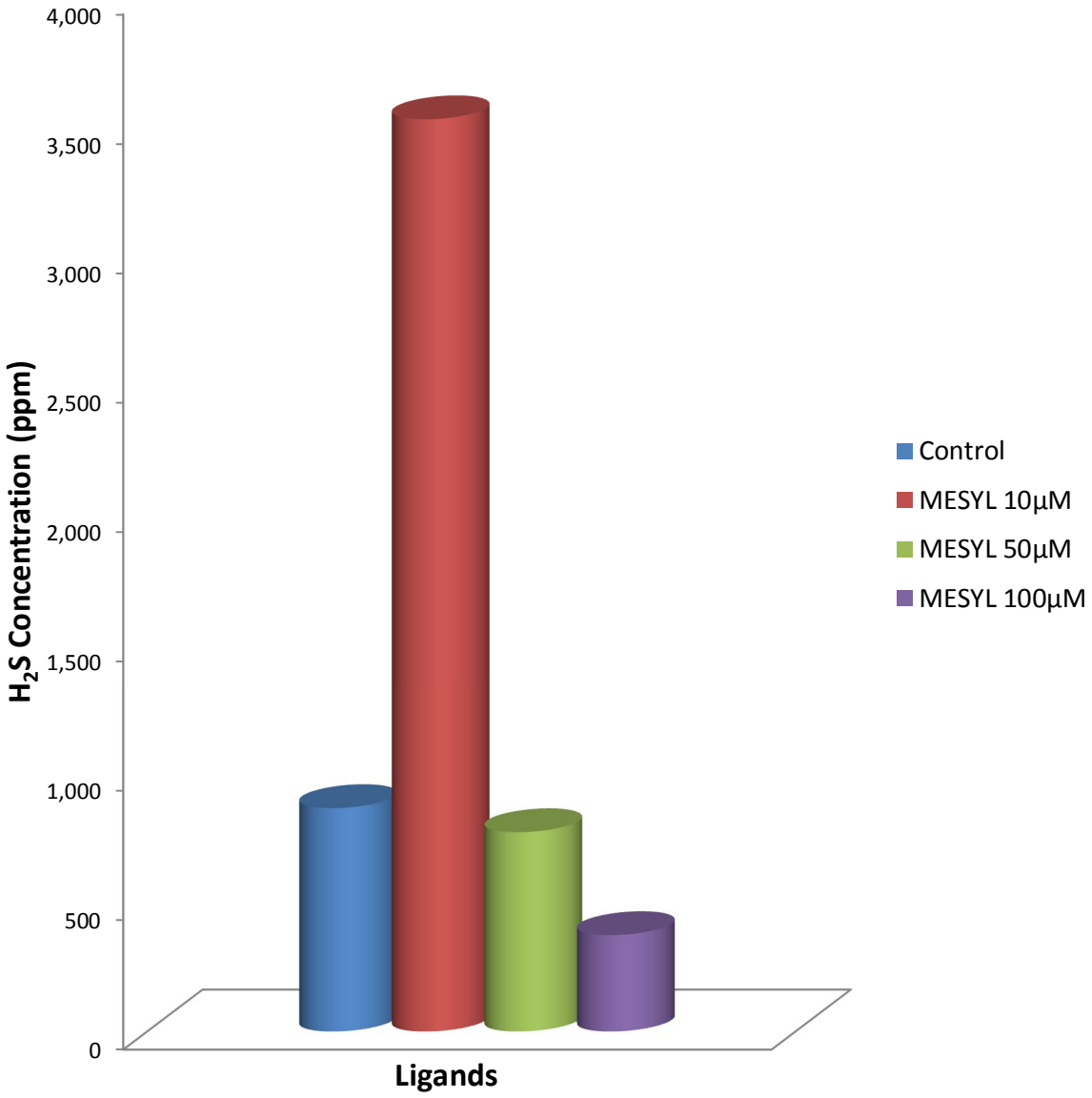


Figure 4.45c: Effect of Increasing MESYL CH:CM concentration on H_2S Concentration

CHAPTER FIVE

5.0 DISCUSSION

5.1 CH_4 gas Yield at Optimized Substrate Weights

The results in Figure 4.1 clearly showed the maximum CH_4 gas yield at optimized substrate weights. All other substrates i.e. GU, BL, CD, and CH, recorded an optimum CH_4 gas yield at optimized weights of 150g except CM which recorded an optimum CH_4 gas yield at 250 g. Therefore it can be concluded that the optimum weight for the substrates investigated in this study is 150 g. This is due to the fact that the CH_4 gas production is highly dependent on the optimum substrate load per system; in other words, there is an optimum feed load per system for a particular size of digester, which will produce maximum gas and beyond which further increase in the quantity of the substrate will not proportionately produce more gas (Mohanrao, 1974). Based on previous studies, our result agrees with expected feed load per digester which is approximately 200 g/L digester (Mohanrao, 1974).

5.2 VFA Concentration of Optimized Substrates

The varying performance of the digester systems seen in Figure 4.1 can be attributed to the VFA concentrations of the systems. Various studies have tried to draw correlation between the concentration of VFA, CH_4 gas production and the health of the anaerobic digester system (Hill and Holmberg, 1988). A well-working biogas digester is therefore characterized by low levels of fermentation intermediates and VFA concentration of less than 2000 mg/L (Hill and Holmberg, 1988). Results showing the VFA concentrations of the digester systems studied are presented in Figure 4.2.

Results from the study showed that the GU digester system recorded a final VFA concentration of 18,499.33 mg/L which was higher than the threshold value of 2000 mg/L. The system continued to experience increasing VFA concentrations as well as decreasing pH and declining CH_4 gas yield. The high VFA concentration brought about instability of the digester system which eventually resulted in the stoppage in production of CH_4 gas.

In the BL digester system, a similar scenario was obtained. The system also recorded a final VFA concentration of 12,207.44 mg/L together with a low pH range of (4.96 – 5.21) (See Table 4.1). The digester system equally showed a steady rise in the VFA concentration which brought about a decline in the CH_4 gas produced.

For the CD digester system, the final VFA concentration (1177.5 mg/L), was significantly lower than the threshold value with an optimum pH ranging from 6.85 – 6.95. This stable biogas system performance was evident in the high CH_4 gas production level observed from the digester system.

In the CM digester system, a high CH_4 gas yield was recorded which was due to the final VFA concentration of 728.61 mg/L and an optimum pH range of 6.54 – 6.77.

For the CH digester system though the VFA concentration was initially high (6618.34 mg/L) and the CH_4 production low, the system finally stabilized with a VFA concentration of 3209.55 mg/L. Though the final VFA concentration for the CH digester is above the threshold value of 2000 mg/L, the system still performed well which was indicated by the high CH_4 gas production and optimum pH range of 6.65-7.30 observed. One possible explanation is the

composition of the microorganism populations present within the digester, which is another factor that affects the performance of a given digester. Thus, VFA concentration cannot be recommended as a stand-alone monitoring parameter.

From the results, the different digester systems showed increasing performance in the order of GU< BL < CD < CM < CH.

5.3 Effect of Substrate Nutrients on CH_4 Gas Yield

Another factor which determines the performance of an anaerobic digester system is the presence of trace metals most especially Fe which serves as nutrients within the digester system. Some works (Marvin, 1989), have reported that Fe is particularly important to the rapid growth of bacteria. The amount of trace metal present in the digesters, as obtained via atomic absorption spectroscopy, is presented in Figure 4.3.

Results from Figure 4.3 show that highest amount of Fe was obtained in samples labelled CH and CD. This further explains the fact that both digester systems recorded high amount of CH_4 gas yield of all the digester systems discussed in this study (Figure 4.1).

Also, Mosey *et al.,* (1971) reported that bioavailability of the necessary nutrients (i.e. the availability for microbial uptake and growth) within the substrates is controlled by the total metal concentration. High concentrations of some of these trace metals like cobalt (Co) and nickel (Ni) usually have an inhibitory effect on the anaerobic digester system. Thus, the presence of these metals ions within the minimum acceptable range is necessary to enhance CH_4 gas production. Results from Figure 4.3 show the presence of these metal ions at the acceptable range

(0.001- 0.050 mg/L) for samples taken out of the CM digester system. This further explains the high CH_4 gas yield obtained within the system. The low performance observed in the GU and BL systems could be attributed to the high VFA concentrations recorded in these systems where VFA concentrations were well above the threshold value of 2000 mg/L (See Figure 4.2). And the fact that some of these metals though present within optimum range, may be in an insoluble form and therefore, unavailable to the Methanogens present.

Apart from the presence of trace metals, other parameters like volatile solid (VS), moisture content, ash content, total solid and the carbon / nitrogen (C/N) ratio greatly influences the production of biogas within digester systems (Kayhanian, 1999; Wang *et al*., 2012). Table 4.2 shows a high C/N ratio in GU and BL digester systems. Low nitrogen content limits microbial activity because microbes need a considerable amount of nitrogen to maintain growth (Zhu, 2010). Therefore, the high C/N ratio in GU and BL indicates a low amount of nitrogen which implies rapid nitrogen consumtion by the methanogens, leading to the low CH_4 gas yield shown in Figure 4.1. Whereas, in CM, CD and CH digester systems, a lower C/N ratio was recorded implying higher nitrogen content; which was indicated in the high CH_4 gas production. The measure of the organic matter content of waste is defined as VS. The higher the VS content, the higher the CH_4 gas produced (Demirel and Yenigun, 2002). From Table 4.2, the % VS was high for CH, BL CM and CD substrate which was indicated by the High % CH_4 gas yield in their substrate digesters (Figure 4.1). Although the % VS for BL was high, its CH_4 gas yield was quite low, implying that other factors invariably contribute to the overall CH_4 gas yield from a digester system. Moisture content is the measure of the amount of water present in a given

sample. The water content is essential for the activities of the anaerobes because the methanogenic activity would decrease with a decrease in the moisture content. From our result, the moisture content value is not so informative with regards to CH_4 gas yield, as all the samples contained equivalent moisture content values. Ash content and Total solid values do not really affect total CH_4 gas yield as much as the other parameters (Demirel and Yenigun, 2002).

5.4 HRT of Optimized Substrates

The digester systems were placed under similar conditions; however each began significant production of CH_4 gas on a different day. The GU digester system in Figure 4.4 began significant production of CH_4 gas on the 22nd day and attained its peak on the 33rd day. Afterwards, production began to drop till it came to a stop on the 49th day. The BL digester in Figure 4.5 began significant CH_4 gas production on the 27th day; percentage of the CH_4 gas continued to rise until it got to its peak on the 36th day, thereafter, production began to decline till it finally came to a stop on the 49th day. For the CD digester (Figure 4.6), significant CH_4 gas production began on the 21st day. The yield of CH_4 gas began to increase till it got to its peak on the 28day; afterwards, it began to drop picked up again on the 31st day, continued to rise and drop till it finally stopped production on the 52nd day. The CM digester in Figure 4.7 began production of methane gas in earnest on the 14th day. The percentage of CH_4 gas continued to rise till it got its peak on the 36th day. Afterwards, there was a decline in the CH_4 gas yield till production finally stopped on the 50th day. Lastly, the CH digester in Figure 4.8 began noticeable production of CH_4 gas on the 19th day and rose to its peak on the 45th day. Thereafter, production began to decline till the system

stopped producing on the 57th day. Thus, the HRT for the digester systems in this study ranged from 49-57 days.

These results for the HRT of the digester systems are in consonance with findings by some other works (Angelidaki and Sanders, 2004) who obtained HRT ranging from 40-50 days for systems operated at mesophilic temperature range. The peak period for each of the five digester systems is affected by the pH values and VFA concentrations within the systems at those periods. Faster start-off periods observed in Figures 4.6 – 4.8 in the CD and CM digester systems are equally due to the lower VFA concentrations and optimum pH values observed within these systems (Figure 4.2 and Table 4.1). The production of CH_4 gas was equally observed to have stopped at different periods. The digester systems GU and BL had the shortest HRT because CH_4 gas production was stalled due to the very high VFA acid concentrations recorded. While for the CD, CM and CH digesters, the HRT indicated periods of complete exhaustion of the organic materials present within each digester system.

5.5 Optimization of Chelating Ligand Ability

The addition of the chelating ligands EDDA, NTA, EDTA, DTPA, PORPH, MESYL and PENI in the digestion systems of the best three performing substrates CM, CD and CH, brought about an increase in the production of methane gas. Depending on the chelating ligand added and the substrate, an increase which ranged from 1-15% in the CH_4 gas production was recorded and presented in Figures 4.9 – 4.11.

With the CM-substrate, the addition of chelating ligands resulted in 2 - 15% improvement in methane production for NTA, EDDA and DTPA (see Figure

4.9). The NTA performed best with a 15% improvement in CH_4 gas production (72.2% CH_4 yield) over the control with a CH_4 gas yield of 64.4%. This was followed closely by the EDDA which showed a 12% increase in CH_4 production (70.5% CH_4 gas yield) and then DTPA with 2% improvement in CH_4 production (65.6% CH_4 gas yield) over the control. All other chelating ligands produced lower percentages of CH_4 gas when compared with the control.

For the CD substrate, addition of chelating ligands resulted in a 1 – 5% improvement in methane production. The EDTA resulted in 5% improvement in methane production (70.3% CH_4 gas yield), followed by the PORPH which showed only 1% improvement in CH_4 production (67.6% CH_4 gas yield) over the control (67.4% CH_4 gas yield) (Figure 4.10) while the DTPA chelating ligand recorded a reduction in CH_4 gas production (66.95% CH_4 gas yield) when compared to the control (67.6%).

With the CH-substrate, the addition of chelating ligand resulted rather in a decrease in CH_4 production (Figure 4.11). The highest percentages of CH_4 produced were observed in MESYL, followed by EDDA and then DTPA with percentage of 69.40%, 57.25% and 24.50% respectively; which were relatively lower than that of the control (84.4%).

For the CM-substrate in Figure 4.12, the final VFA concentrations for EDDA and NTA digester systems were given as 476.05 mg/L and 671.96 mg/L respectively. For DTPA, a final concentration of 737.63 mg/L was equally recorded. This low VFA concentration levels in EDDA, NTA and DTPA is implicated in the high CH_4 gas yield observed within these digester systems. All other chelating ligands (EDTA, PORPH, PENI, and MESYL) showed higher

peak concentration of VFA when compared with the control; their values were 1451.20, 1058.83, 686.92, and 949.49 mg/L respectively. In general, the VFA concentrations were significantly lower than 2000 mg/L with pH range between 6 – 7.5. (`Table 4.3), indicating that all anaerobic digester systems performed appreciably above average.

With the CD-substrate in Figure 4.13, low VFA concentrations and an optimum pH range of 6.81 – 7.42 (Table 4.3) were observed in all the chelating ligands digester systems when compared with the control. EDDA, NTA, EDTA, MESYL, DTPA, PORPH and PENI had VFA final values of 240.65, 628.51, 483.75, 267.82, 607.39, 127.24, and 311.38 mg/L respectively. This result equally reveals that the digester systems were operating at optimum level; which accounts for the relatively high CH_4 gas yield observed from these digester systems.

For the CH-substrate in Figure 4.14, very high VFA concentrations were reported in all chelating ligand containing systems. EDDA, NTA, EDTA, MESYL, DTPA, PORPH, and PENI had peak VFA concentration values of 14,364.9, 28,112.01, 40,036.56, 15,939.96, 39,069.44, 42,414.98, 40,457.75 mg/L respectively. This accounts for the poor performance and the low CH_4 gas production observed within these systems.

The low stability constant of PENI with Fe, Co and Ni explains why CH_4 production with this chelating ligand is low with all substrates containing it. MESYL forms complexes only with Fe thus giving reason for the low CH_4 production observed in all substrate containing the MESYL chelating ligand except for CH-substrate where Fe^{3+} concentration is high (Figure 4.3).

However, other variables like the number of heterocyclic rings formed in the chelates and relative concentration of the metal ions also contributes to the bioavailability of ions inspite of the higher stability constant (Anderson, 1999). In addition, chelating ligands which form lipophilic complexes may permeate the plasma membrane of an organism (methanogen) and assist in the uptake of metal ions. This explains why the addition of the chelating ligand EDTA and DTPA to the digester systems (which form stable complexes with Fe, Ni and Co) does not bring about a significant improvement in CH_4-production. EDTA and DTPA are poorly absorbed into cells of methanogens which limits their capacity to chelate-out metals from inside the cells of the methanogens (Klaaseen, 2006). This is likely responsible for the not-so significant CH_4-production observed in some digester systems into which they were added; for instance the CD EDTA and CD DTPA digester systems.

Though NTA and EDDA form stable chelates with Fe, Co and Ni as is the case with EDTA and DTPA, their complexes are lipophilic (Hartwig *et al.,* 1993). As a result, NTA and EDDA are able to permeate the membranes of the methanogens allowing them to reach the sites of metal storage and thus form stable complexes with these metal ions thereby leading to the significant improvement in CH_4-production observed within those digesters.

5.6 Effect of Addition of Chelating Ligand on HRT

The HRT for the methane production was equally reduced with the addition of chelating ligands (Figures 4.15a – 4.17c). With the CM substrate, Figures 4.15a-4.15c, the HRT was 50 days for the control but reduced to 25 days on addition of chelating ligands. For all chelating ligand containing systems, significant

production of CH_4 gas was observed between the 1st and 2nd day and production attained its peak between the 8th and 18th day depending on the chelating ligand. Afterwards, there was a decline in CH_4 gas yield till production finally stopped on the 25th day.

For the CD substrate, Figures 4.16a - 4.16c, HRT was 52 days for the control but with the addition of chelating ligands, the time reduced to 30 days. Significant production of CH_4 gas was also observed for all the systems containing chelating ligands 24hours after the experimental setup and attained its peak between the 8th and 24th day. CD substrate continued CH_4 gas production till it came to a stop on the 30th day.

In the case of the CH-substrate, Figures 4.17a – 4.17c, HRT was reduced to 32 days as against the control which had a HRT of 57 days. Significant production of CH_4 gas was observed in all the digester on the 8th day, production continued to improve till the peak value was obtained between the 12th and 22nd day. Thereafter, production of CH_4 gas began to decline till the system finally stopped producing on the 32nd day.

The decrease in HRT is due to the metal chelate catalysis of the hydrolysis stage (which is the rate determining step) of the anaerobic digestion process where the metal chelate formation, which is as a result of the reaction occurring between metals serving as nutrients within the digester system and the chelating ligands introduced; serve as a driving force in solvolysis process (Kroll, 1952). Depending on the content of the waste material fed into a digester system, different stages may be the rate-limiting step in the digestion process. In the digestion of soluble compounds, the rate-limiting step has been identified as the

methanogenesis stage which is the final stage in the anaerobic digestion process. But for the digestion of insoluble wastes, the hydrolytic stage is the rate-limiting stage (Marvin, 1989).

Metal chelate catalysis is considered to include any reaction that is altered or modified by metal ions through chelate ring formation, as well as reactions of the metal chelates themselves (Kroll, 1952). In a solvolysis reaction, the chelated ligand reacts with an electron donating solvent molecule, since the electronic interaction of a ligand with a metal would always increase its reactivity towards nucleophilic reagents. Such large catalytic effects are observed when ligand donor groups are in positions that hold the metal close to the reactive centre, or when the formation of the stable metal chelate is the result of solvolysis reaction (Angelici and Leach, 1968). As is the case in digester systems where metals present react with the chelating ligand to form stable metal chelates. The hydrolysis of amino acids during the process of anaerobic digestion is an example of this type of strongly metal-catalysed solvolysis reaction. Kroll (1952) reported that the alpha amino acid coordinate transition metal ions like Fe and Co, through the amino nitrogen and carbonyl oxygen. The additional polarization of the carbonyl oxygen thus achieved, facilitates nucleophilic attack of the carbonyl carbon by a polar water molecule to produce a number of reactive intermediates in rapid equilibrium, differing in the positions of attachment of protons and the metal ion (Figure 5.1). Further reaction occurs, converting these reactive intermediates into methanogenic substrates like acetate and hydrogen; which proceed to form CH_4, CO_2 and H_2O.

Generally, the catalytic effect of the metal ion parallels the stabilities of the corresponding amino acid chelates, in agreement with the concept that metal

chelate formation is the principal driving force of these reactions. Thus, helping to speed up the whole anaerobic digestion process.

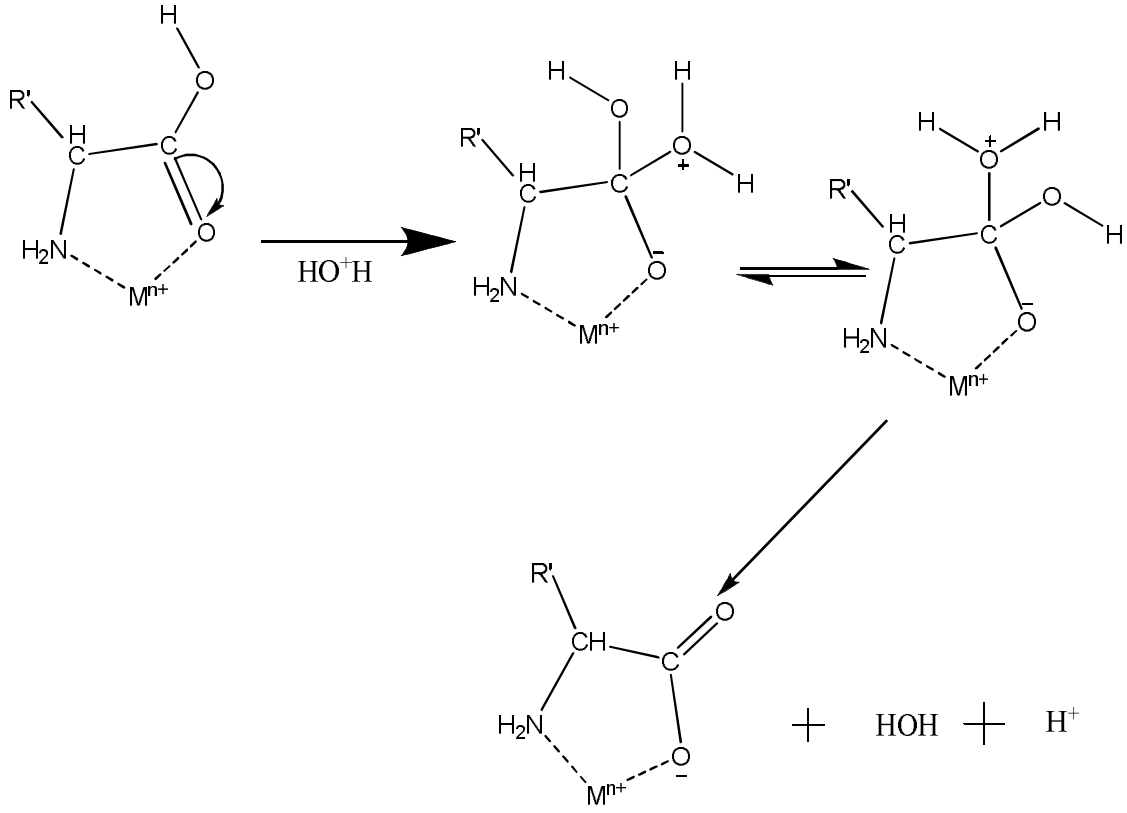


Figure 5.1: Equation of Reaction Showing Catalytic Effect of Metal ions in the Formation of Reactive Intermediates during the course of the Anaerobic Digestion Process.

In summary, stimulation of the digester systems by the addition of chelating ligands, resulted in 12 – 15% improvement with CH_4-production in digester systems containing EDDA, and NTA chelating ligands; while digester systems containing chelating ligands EDTA, PORPH, and DTPA, showed 1 – 5% improvement in CH_4-production over the control. Also, faster digester start-up

reduced HRT and more rapid digester recovery from upset resulted from the addition of chelating ligands.

5.7 Effect of Increasing Concentration of Chelating Ligand on CH_4 gas yield

Although addition of the chelating ligand did improve CH_4-production, nevertheless, a decrease in CH_4-production was observed on increasing the chelating ligand concentrations within digester systems. From Figure 4.18a, it was observed that the CM digester system containing EDDA chelating ligand at a concentration of 10 μM, produced 70.50% CH_4 gas; but on increase of the EDDA concentration to 50 μM, the amount of CH_4 gas produced reduced to 52.10%; and a further increase in the EDDA concentration further brought the yield of CH_4 down to 43.40%. Also, in Figure 4.18b, on increasing the NTA chelating ligand concentration from 10 μM to 50 μM, the CH_4 gas yield dropped from 72.20% to 43.10% and further increase in NTA concentration did not improve the CH_4 gas yield.

For the CD substrate (Figure 4.19a), increasing the EDTA concentration from 10 μM to 50 μM resulted in a decrease in the CH_4 gas production from 70.30% to 43.70%. Also, increasing the DTPA and PORPH (Figure 4.19b and Figure 4.19c), chelating ligand concentration further reduced the CH_4 gas yield from 66.90% to 54.20% and 67.40% to 59.50% for DTPA and PORPH respectively.

The different scenario was observed in the CH substrate on increase in EDDA, DTPA and MESYL chelating ligand concentration (Figure 4.20). Increasing the chelating ligand concentration from 10 μM to 100 μM increased the CH_4 gas yield from 57.20% to 63.10% for EDDA (Figure 4.20a); in the case of DTPA (Figure 4.20b), the gas yield equally increased from 24.50% to 75.30%. But for

MESYL (Figure 4.20c), increasing chelating ligand concentrations from 10 μM to 100 μM brought about a decrease in CH_4 gas yield from 69.40% to 19.80%.

5.8 Effect of Addition of Chelating Ligand on H_2S Concentration

The presence of H_2S in biogas at a concentration above 1000 ppm is a major issue in the biogas industry (Gary, 2003). Thus, reduction in its concentration is imperative for the production of high quality biogas.

Figures 4.21 – 4.23 show the effect of addition of chelating ligand on H_2S concentration in the CM, CD and CH digester systems. The results showed a general decrease in H_2S concentration from 80 – 30% on addition of chelating ligands at a concentration of 10 μM in the CM, CD and CH substrate digesters. On further increase in the concentration of the chelating ligand, a noticeable decrease in the H_2S concentration by about 20% is equally observed in all the digester systems. This implies that the addition of chelating ligands has served as a scrubber; helping to reduce the concentration of H_2S and equally improve the quality of the CH_4 gas produced.

A redox reaction process is responsible for the desulphurisation observed within the digester systems. Metals (e.g. Fe, Co, and Ni) serving as nutrients already present within the systems, react with the various chelating ligands introduced, to form metal chelates. These metal chelates then react with the H_2S gas produced, absorbing the gas from the system. The reaction sequence for the absorption of the H_2S by the metal chelates is presented in the equation below:

$$H_2S_{(g)} \leftrightarrow H_2S_{(aq)} \quad 5.1$$

$$H_2S_{(aq)} + M^{x+}\,Chelant^{y-} \rightarrow S\downarrow + 2H^{+} + 2M^{(x-1)}\,Chelant^{y-} \quad 5.2$$

Where; "x" denotes the charge of the metal cation.

"y" denotes the charge of the chelant anion and

"M" represents the metal ion.

In the absorption reaction, H_2S comes in contact with the metal chelate to bring about the reduction of M^{n+} to $M^{(n-1)+}$ and the oxidation of H_2S to form elemental sulphur with the release of H^{+} ions; thus the concentration of H_2S gas produced within the digesters is thereby reduced (Gary, 2003).

5.9 Effect of Increasing Chelating Ligand Concentration on VFA

For the VFA concentration, increasing the chelating ligand concentration equally increased the VFA concentrations within digester systems which accounts for the low system performance and low CH_4-production observed in the result (Figures 4.24 – 4.26).

In the CM digester system (Figure 4.24), increasing the chelating ligand concentration from 10 µM to 50 µM equally increased the VFA final concentration from 476.05 to 2112 mg/L, for EDDA (Figure 4.24a) and 737.63 to 1013.0mg/L for DTPA (Figure 4.25c). However for NTA (Figure 4.24b), the VFA concentration rather decreased from 671.96 to 561.30 mg/L. Further increase in the chelating ligand concentration from 50 µM to 100 µM, equally increased the VFA concentration for all the CM digester systems.

For the CD digester system (Figure 4.25), increasing chelating ligand concentration from 10 μM to 100 μM increased the VFA concentration from 483.75 to 1892.19 mg/L, 127.24 to 1377.61 mg/L, and 607.39 to 1773.17 mg/L for EDTA (Figure 4.25a), PORPH (Figure 4.25c) and DTPA (Figure 4.25b)respectively.

With the CH digester systems (Figure 4.26a – 4.26c), increasing chelating ligand concentration rather brought about a decrease in VFA concentration in some of the chelating ligand systems. By the increase in chelating ligand concentration from 10 μM to 100 μM, the VFA concentration reduced from 9204.55 to 7027.10 mg/L for EDDA (Figure 4.26a). In the case of DTPA (Figure 4.26b), the concentration was observed to have dropped from 39,069.44 to 11,048.37 mg/L and for MESYL (Figure 4.26c), the VFA concentration decreased from 9277.92 to 9141.41 mg/L. This decrease in the VFA concentration is accounted for by the increase in CH_4 gas yield observed in all the digester systems. Except MESYL where a decreasing VFA concentration resulted in a decrease in the percentage of CH_4 gas produced. The decrease in CH_4 gas produced MESYL 100μM is attributed to the composition of microorganism population present within the digester at that concentration.

The amount of VFA produced is the major cause of a decline in the pH and percentage CH_4 produced within the digester systems (Jain and Mattiasson, 1998). This increase in the VFA concentration observed in CM and CD digester systems could be explained from equation (5.2) where the absorption reaction process between H_2S and metal chelates formed, leads to the production of H^+ ions. Therefore, the higher the concentration of the chelating ligand, the higher the H^+ ions (total proton-donating capacity) present within the digester system;

leading to an increase in the VFA concentration, an eventual decrease in pH and reduction in CH_4 gas yield (Figure 18a-19c).

For the CH digester systems, a high percentage of H_2S gas is observed in the systems lacking chelating ligands (i.e. the control). On the addition of chelating ligands, a reduction in VFA concentration was observed which was due to the decrease in the percentage of H_2S available within the systems (Figure 4.23c). The decrease in the H_2S%, eventually lead to a decrease in the amount of H^+ produced from the reaction of H_2S with the chelating ligands (equation 5.2) when compared to the control. H_2S is toxic to methanogens and to the environment together (Winfrey and Zeikus, 1977). Thus, reduction in the concentration of H_2S invariably improves the population of methanogens; thereby, leading to the increased CH_4 gas yield observed within the CH digester (Figure 20a-b).

5.10 Effect of Increasing Concentration of Chelating Ligand on HRT

A study on the effect of increasing chelating ligand concentration on HRT was equally carried out and the results presented in Figures 4.27 – 4.35. A further decrease in HRT is observed on increase in chelating ligand concentration. For the CM substrate (Figures 4.27 – 4.29), in all the digester systems, increasing chelating ligand concentration from 10 μM to 50 μM and then to 100 μM, reduced the HRT from 25 days to 19 days. For the CD substrate (Figure 4.30–4.32), increasing chelating ligand concentration from 10 μM to 100 μM also reduced HRT from 30 to 25 days. A slight reduction in HRT was also observed in the CH substrate (Figure 4.33 – 4.35) from 32 days to 30 days on increase in Chelating ligand concentration. These observations equally points to the metal

chelate catalytic nature earlier discussed [i.e. the decrease in HRT due to the metal chelate catalysis of the hydrolysis stage which is the rate determining step of the anaerobic digestion process; where the metal chelate formation is the driving force in the solvolysis process (Kroll, 1952)]. This process speeds up the hydrolytic stage of the anaerobic process thus, reducing the HRT.

5.11 Co-digestion

From the results of the Co-digestion analysis recorded in Figure 4.36, the CH: CM blend was seen to have performed best with a maximum CH_4 gas yield of 78.8% on the 17^{th} day. All other blends performed poorly with peak gas yields ranging from 3.9 – 23.3%. CH: GU had a maximum gas yield of 23.3%, followed by CM: GU with a maximum gas yield of 14.2%. Next was CM: BL with a maximum gas yield of 8.7%, then CH: BL with a maximum gas yield of 6.3% and lastly GU: BL with a maximum gas yield of 3.9%. Table 4.2 shows high carbohydrate (CHO) content in systems mixtures containing GU, BL and CH. This high carbohydrate content is equally responsible for the increasing digester acidity within the digester system (Winfrey and Zeikus, 1977). And from earlier discussions, high H^+ ion concentrations leads to increased VFA levels and an ultimate decrease in the system performance bringing about reduction in the CH_4 gas production observed within the CH: GU, GU: BL, CH: BL, CM: BL and CM: GU digester systems.

5.12 Effect of Chelating Ligand on CH_4 gas Yield and VFA Concentration in CH: CM Co-digestion System

From Figure 4.37,the addition of the different chelating ligands to the co-digester systems resulted in 3% improvement in CH_4-production observed in the EDDA digester system (79.6%) compared to the control which recorded are CH_4

gas yield of 78.8%. DTPA showed 73.6% CH_4-production, MESYL 71.0%, EDTA 70.1% and NTA 68.2% CH_4-production. For the other digester systems, CH_4 gas production was below 60%. Thus, from the result it shows that little or no improvement is indicated in co-digestion systems containing chelating ligands except for the digester system with EDDA. This is due to the high lipophilic complexing ability of EDDA chelating ligand compared to the other chelating ligands (Hartwig *et al*., 1993).

Figure 4.38, showed the effect of addition of chelating ligand on the VFA concentration. All digester systems containing chelating ligands showed VFA concentration higher than 2000mg/L. The EDDA, DTPA, MESYL, EDTA, NTA, PENI and PORPH recorded final VFA concentrations of 4362.35, 5128.39, 9324.56, 2378.50, 9779.10, 4827.76 and 1529.41mg/L respectively. The concentration of VFA produced, which if it exceeds the threshold value of 2000mg/L; is the major cause of decline in the percentage yield of CH_4 gas (Jain and Maitiasson, 1998). This therefore accounts for the low CH_4 gas yield and poor system performance observed in Figure 4.37.

5.13 Effect of Increasing Concentration of Chelating Ligand on CH_4 gas yield and VFA Concentration in Co-digestion

Figure 4.39, shows the effect of increasing chelating ligand concentration on CH_4-production in co-digestion systems. Increasing the chelating ligand concentration from 10µM to 100µM for the EDDA brought about a decrease in the CH_4 gas yield from 79.6% to 71.9%, and for DTPA, MESYL, EDTA, and NTA, there was a general decrease in the CH_4 gas yield by about 5%. Thus, indicating that an increase in the chelating ligand concentration did not equally improve the CH_4-production in the co-digestion systems.

Figure 4.40, shows the effect of increasing chelating ligand concentration. The results equally revealed VFA concentrations higher than 2000mg/L; though the increase in the chelating ligand concentration from 10 to 100μM resulted in reduction in the VFA concentration. EDDA, DTPA, and MESYL chelating ligand co-digestion systems recorded VFA concentrations of: 2808.38, 2786.82 and 2544.16 mg/L respectively.

The percentage of CH_4-produced observed in all the co-digester systems containing increased concentration of chelating ligands was quite low (Figure 4.39); implying that an increased chelating ligand concentration did not improve CH_4 gas yield. The only improvement of about 1% observed in the EDDA digester system at 10μM was due to the increased bioavailability of essential inorganic nutrients on addition of Chelating ligands which invariably promoted the growth and stability of the methanogenic population thereby improving CH_4 production. Thus, the addition of chelating ligands to co-digestion systems did not lead to a significant improvement in the CH_4 gas yield.

5.14 Effect of Addition of Chelating Ligand on HRT in Co-digestion

The HRT for the methane production was observed to have equally reduced with the addition of chelating ligands (Figures 4.41a -4.41c). The HRT which was 35 days for the control, reduced to about 26 days on addition of the different chelating ligands. Significant production of CH_4 gas was observed on the 2^{nd} day; production continued until the peak was attained between the 19^{th} and 22^{nd} day depending on the chelating ligand. Afterwards, there was a decline in the CH_4 gas yield till the production finally came to a stop on the 26^{th} day.

A further decrease in HRT from 26days to 21days was equally recorded on increasing in chelating ligand concentration from 10µM to 100µM (Figure 4.42 – 4.44). This decrease in the anaerobic reaction process was equally due to the catalytic nature exhibited by metal chelates formed on addition of the chelating ligands; thereby increasing rate of the hydrolysis stage (Kroll, 1952).

5.15 Effect of Chelating Ligand on H_2S Concentration in Co-digestion

To the CH: CM co-digester system, increasing concentrations of the best chelating ligands EDDA, DTPA and MESYL were added (Figure 4.45). It was observed that no significant improvement in the scrubber ability of each chelating ligands was observed when introduced at low concentrations of 10µM into the co-digestion systems compared with the control. However, increasing the concentration of the chelating ligand to 50µM brought about a reduction in the concentration of the H_2S by about 80%. And a further increase in concentration to 100µM further reduced the H_2S concentration by about 10%. Thus indicting that, the addition of chelating ligand had a positive effect in reducing the concentration of H_2S within the digester systems. As earlier explained, this observation is equally due to the redox reaction process taking place within the co-digestion system on addition of chelating ligands; resulting in desulphurisation due to the formation of metal chelates.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

The results for the analyses carried out in this research showed that, at mesophilic temperature range of 29 – 31^{o}C, four of the different substrates, GU, BL, CH and CD, recorded maximum CH_4 gas yield of 8.30%, 33.30%, 84.80% and 67.60% respectively at an optimum weight of 150g. The only exception observed was with CM substrate which recorded a CH_4 gas yield of 75.40% at an optimum weight of 250 g. The result agrees with the expected feed load per digester which is approximately 200 g/L digester.

On addition of chelating ligands, EDDA, NTA, EDTA, DTPA, MESYL, PORPH and PENI, it was observed that depending on the chelating ligand added and the substrate, an increase in the CH_4 yield was recorded which ranged from 1-15%. This improvement in CH_4 gas yield was attributed to the increased bioavailability of essential nutrients on addition of chelating ligands which invariably promoted the growth and stability of the CH_4 producing bacteria; thereby improving CH_4 production.

Also, a general reduction in HRT, from about 50 days to 25 days, was observed in all digester systems containing chelating ligands. The decrease in HRT was due to the metal chelate catalysis of the hydrolysis stage of the anaerobic digestion process were the metal chelate formation served as the driving force in the solvolysis process.

The effect of increasing chelating ligand concentration on production of H_2S was also studied. And the result showed a general decrease in H_2S concentration from 30% to 80% on increasing the chelating ligand concentration within the

best three performing substrate digesters namely, CM, CD and CH, from 10 µM to 100 µM. This reduction was due to the redox reaction occurring between the various metal chelates and H_2S, leading to the formation of elemental Sulphur with the release of H^+ ions; thus reducing the concentration of H_2S within the digester systems. Although increasing the concentration of chelating ligands reduced the percentage of H_2S, a decrease in CH_4 gas yield was observed in the process.

For the CM digester system containing EDDA chelating ligand, increasing chelating ligand concentration from 10 µM to 100 µM resulted in a decrease in the CH_4 gas yield from 70.50% to 43.40%. Also, increasing NTA chelating ligand concentration from 10 µM to 50µM reduced the CH_4 gas yield from 72.2% to 43.70%. For the CD substrate increasing EDTA concentration from 10 µM to 50 µM brought about a reduction in the CH_4 gas yield from 70.30% to 43.70%; also, increasing the DTPA and PORPH chelating ligand, reduced the CH_4 gas yield from 66.90% to 54.20% and 67.40% to 59.50% respectively. Except for the CH digester system, where increasing the EDDA and DTPA chelating ligand concentration brought about increase in the CH_4 gas yield from 24.50% to 75.30% for DTPA and 57.20% to 63.10% for EDDA. But for MESYL chelating ligand, increasing concentration lead to a reduction in CH_4 gas yield from 69.40% to 19.80%. The poor system performance recorded within CM and CD digester systems is due to the increasing VFA concentration observed on increase in chelating ligand concentration; which is as a result of the increased H^+ ion concentration as H_2S reacts with the metal chelates formed.

A further decrease in HRT was observed on increasing chelating ligand concentration. For the CM substrate, increasing chelating concentration from 10

μM to 100 μM reduced the HRT from 25 to 19 days. For the CD substrate, increasing chelating ligand concentration from 10 μM to 100 μM reduced HRT from 30 to 25 days. Also, in CH substrate, a slight decrease in HRT from 32 to 30 days was observed on increasing chelating ligand concentration. These findings are equally due to the catalytic nature observed on formation of metal chelate within the digester systems.

The effect of co-digestion was also studied and the results showed that the addition of EDDA chelating ligand resulted in a 3% improvement in CH_4 gas yield. For the other digestion systems, no noticeable improvement in CH_4 production was observed. Also, increasing the concentration of EDDA chelating ligand brought about decrease in the CH_4 production from 79.605 to 71.9%. And for the other chelating ligands, a general decrease in CH_4 gas yield of about 5% was recorded on increasing chelating ligand concentration. The high VFA concentrations recorded within the digester system on addition of (and equally increasing) chelating ligand concentrations accounts for the poor performance and low CH_4 production observed.

6.1 Conclusion

This work has established the optimum percentage of CH_4 gas yield at optimized substrate weights for GU, BL, CD, CM, and CH as 8.30%, 33.30%, 67.40%, 75.40% and 84.80% respectively. This research has employed the use of chelating ligands EDDA, NTA, DTPA, EDTA, MESYL, PENI and PORPH for the improvement of CH_4 gas yield in CM, CD and CH digester systems for the first time. In this work, the use of chelating ligands EDDA, NTA, DTPA, EDTA, MESYL, PENI and PORPH has also been employed to reduce HRT in CM, CD, and CH anaerobic digesters for the first time.

This research has established that, the use of chelating ligand EDDA, NTA, DTPA, EDTA, MESYL, and PORPH is effective in reducing the concentration of H_2S gas in CM, CD, and CH anaerobic digesters from 30-80%. This work also reports for the first time that the use of EDDA chelating ligand on CH: CM co-digestion system, improves the CH_4 gas yield of the system by 3%. Lastly, in terms of contributions to the production of CH_4 gas, EDDA, NTA, DTPA, EDTA and MESYL have been most effective in improving the percentage yield of CH_4 gas in CM, CD, and CH anaerobic digester systems.

Thus, the use of chelating ligands has been shown to be a promising and sure way of improving both quality and percentage of CH_4 gas produced from solid waste digester systems.

6.2 Recommendation

Further studies should be carried out to determine:

- The effect of the addition of chelating ligands and enzymes to liquid waste samples (sludge) on the growth rate of wide variety of methanogens, CH_4 gas yield, HRT and concentration of H_2S.

- The effect of Ultrasonic and Pulse power pre-treatment methods on digester systems as all these may generate biogas as well.

REFERENCES

Ahn, H.K., and Smith, M.C.(2008). Biogas Production Potential from Switch Grass-Animal Manure Mixture using Dry Anaerobic Digestion. *In: Proceedings of American society of agricultural and biological engineers annual international meeting*. June 29- July 02, Providence, Rhode Island, 12: 7317-7326.

Al Seadi, T. (2001). Good Practice in Quality Management of AD Residues from Biogas Production. *Report made for the International Energy Agency, Task 24-Energy from Biological Conversion of Organic Waste*. Published by IEA Bio-energy and AEA Technology Environment, Oxfordshire, United Kingdom, pp 53-57.

Amani, T., Nosrati, M., Sreekrishnan, T.R. (2011). A Precise Experimental Study on Key Dissimilarities Between Mesophilic and Thermophilic Anaerobic Digestion of Waste Activated Sludge. *International Journal of Environmental Resources*. 5(2): 333-342.

Anderson, G.K., Kasapgil, B. and Ince, O. (1994). Microbiological Study of Two-Stage Anaerobic Digestion During Start-Up. *Water Research*, **28**, 2383-2392.

Anderson, R.P. (1999). Preliminary Review of the Systematics and Biogeography of The Spiny Pocket Mice (Heteromys) of Colombia. *Revista de la Academia Colombiana de Ciencias Exactas, Fisicas y Naturales,* 23 (suplemento especial), 613-630.

Andreiadis, E. S., Chavarot-Kerlidou, M., Fontecave, M., Artero, V. (2011). Artifical Photosynthesis: From Molecular Catalysts for Light-driven Water Splitting to Photoelectrochemical Cells. *Photochemistry and Photobiology* **87** (5): 946–964.

Angela, M.R., Kevin, M.R., Tracy, L.H. (2012). Predicting Litter and Live Herb Fuel Consumption during prescribed Fires in native and old-field Upland Pine Communities of the Southeastern United States. *Canadian Journal of Forest Research*, 42(8):1611-1622.

Angelici, R.J., Leach, B.E. (1968). Reactions of Metallic Salts. *Journal of inorganic Chemistry,* 11 p203.

Angelidaki, I.,and Sanders W. (2004). Assessment of the Anaerobic Biodegradability of Macropollutants. *Reviews in Environmental Science and Biotechnology*. 3:117–129.

A.O.A.C. (*1980*). Official Methods of Analysis, 15th ed. Association of Official Analytical. Chemists. Washington D.C. 376-384.

Attar, Y., Mhetre, S.T. and Shawale, M.D. (1998). Biogas Production Enhancement by Cellulytic Strains of Actinomycetes. *Biogas Forum I* (72), 11–15.

Awosolu, M.O. (2007). *Anaerobic Digestion of Ethanol Distillery Waste-Stillage for Biogas Production,* M.Sc. Thesis, University of Hogskolani Boras, Sweden, pp 5-15.

Baier, U.and Schmidheiny, P. (1997). Enhanced Anaerobic Degradation of Mechanically Disintegrated sludge .*Water Science and Technology*.36 (11),137–143.

Batstone, D.J., Keller ,J., Angelidaki, I., Kalyuzhnyi S.V., Pavlostathis, S.G,,Rozzi, A, Sanders, W.T.M., Siegrist, H.,Vavilin, V.A. (2002). Anaerobic Digestion Model No.1. IWA Task Group on Mathematical Modelling of Anaerobic Digestion Processes. *IWA Scientific and Technical Report*. No. 13, IWA Publishing, London, UK, pp11-12.

Bhattacharya, T.K. and Mishra, T.N.,(2003). Biodegradability of dairy cattle manure under dry anaerobic fermentation process. *Journal of the Institution of Engineers (India): Agricultural*. Engineering. Division., 84: 9-11.

Braber, K. (1995). Anaerobic digestion of municipal solid waste: a modern waste disposal option on the verge of breakthrough. *Biomass Bioenergy*. 9 (1-5): 365-376.

Brian R, (2000). Chemistry 1. Cambridge University press. ISBN 0-521-78778-5.

Brummeler, E.T. (2000). Full scale experience with the BIOCEL process. *Water Science and Technology*, 41, 299-304.

Buratti, C., Costarelli, I., Cotana, F., Crisostomi, L. and Fartozzi, F. (2005). The Biomass Research Centre for Biomass Characterization*, European Biomass Conference and Exhibition*, Paris, France, pp 6-9.

Callander, I.J. and Barford, J.P. (1983). Recent Advances in Anaerobic Digestion Technology. *Process Biochemistry*, 18, 24 - 30.

Chen, Y., Cheng, J.J, and Creamer, K.S. (2008). Inhibition of Anaerobic Digestion Process: A Review. *Bioresource Technology*, 99: 4044–4064.

Cho, J.K., Park, S.C. and Chang, H.N. (1995). Biochemical Methane Potential and Solid State Anaerobic Digestion of Korean Food Wastes. *Bioresource Technology*, 52(3): 245-253.

Chugh, S., Chynoweth, D.P. and Clarke, W. (1999). Degradation of Unsorted Municipal Solid Waste By A Leach-Bed Process. *Bioresource Technology*, 69(2): 103–115.

Cohen, A., Breure, A.M., van Andel, J.G., and van Deursen, A. (1982). Influence of Phase Separation on the Anaerobic Digestion of Glucose. ii. Stability and Kinetic Responses to Shock Loadings. *Water Research*, **16**, 449 - 455.

Dar, H.G.,and Tandon, S.M., (1987). Biogas Production from Pretreated Wheat Straw, Lantana Residue, Apple and Peach Leaf Litter with Cattle Dung. *Biological Wastes*. 21, 75–83.

Demirel, B., and Yenigun, O. (2002). Two- phase anaerobic digestion Processes: a review. *Journal of Chemical Technolgy and Biotechnology,* 77 (7), 743-755.

Dieter, D. and Angelika S. (2008). *Biogas from Waste and Renewable Resources.* SNP Best-Set Typesetter Ltd., Hong Kong, pp. 93 – 154.

Dhawale, M.R. (1996). Anaerobic Fermentation with Chemical Inducers and Higher Solids for Biogas Production. Project Sponsored by MNES, 1993–1996, *Shivasadan Renewable Energy Research Institute.* Sangli, Maharashtra, India, p 6.

Edelmann, W. (2003). Biomethanization of The Organic Fraction of Municipal Solid Wastes. In: *Mata-Alvarez, J. (Ed.).* IWA Publishing, London. pp.265–301.

Elefsiniotis, P. and Oldham, W.K. (1994) Anaerobic Acidogenesis of Primary Sludge: the Role of Solids Retention Time. *Biotechnology and Bioengineering*, 44, 7-13.

Fatma, A., Yutaka, N. and Naomichi, N. (2009). Dry Mesophilic Fermentation of Chicken Manure for Production of Methane by Repeated Batch Culture. *Journal of Bioscience and Bioengineering*., 107(3): 293–295.

Field, C.B., Campbell, J. E.,and Lobell, D. B. (2008). Biomass Energy: The Scale of the Potential resource. *Trends in Ecology Evolution* 23: 65–72.

Fletcher Jr., R. J., Bruce, A. R., Jason, E., Patrick, J. D., Janaki, R. R. A., Douglas, W. S. (2011). Biodiversity conservation in the era of biofuels: risks and opportunities. *Frontiers in Ecology and the Environment.* **9** (3): 161–168.

Fraser, M. D. (1977). The Economics of SNG Production by Anaerobic Digester of Specially Grown Plant Matter. *Proceedings Symposium Fuels from Biogas and Waste.* Institute of Gas Technology, Chicago.,p 7

Foresti, E. (2001). Perspective on Anaerobic Treatment in Developing Countries. *Water Science Technol*ogy. 44:141-148.

Fox, P. and Pohland, F.G. (1994). Anaerobic Treatment Applications and Fundamentals: Substrate Specificity During Phase Separation. *Water Environment Research*, **66**, 716-724.

Gary J. N. (2003). Removing H_2S from Gas Streams, Report by U.S. Filter Gas Technology Products, USA, p 5.

Geeta, G.S., Suvarna, C.V. and Jagdeesh, K.S.(1994). Enhanced Methane Production by Sugarcane Trash Pretreated with Phanerochaete Chrysosporium. *Journal of Microbiology Biotechnology*.9 (2), 113–117.

Ghaly, A.E., 1996. A Comparative Study of Anaerobic Digestion of Acid Cheese 72. Whey and Dairy Manure in a two-stage Reactor. Bioresource Technology.58 (1), 61–65.

Ghosh, S. and Pohland, F.G. (1974). Kinetics of Substrate Assimilation and Product Formation in Anaerobic Digestion. *Journal of Water Pollution Control and Federation*, 40, 748-759.

Ghosh, S. (1990). Principles and Potentials of Biphasic Fermentation. *Biogas Technologies and Implementation Strategies International Conference*, 473-497.

Ghosh, S., Henry M.P., Sajjad A., Mensinger M.C. and Arora J.L. (2000). Pilot-Scale Gasification of Municipal Solid Wastes by High-Rate and Two-Phase Anaerobic Digestion (TPAD). *Water Science and Technology*, 41, 101-110.

Guendouz, J., Buffière, P., Cacho J., Carrère, M.and Delgenes ,J.P. (2010). Dry Anaerobic Digestion in Batch Mode: Design and Operation of A Laboratory-Scale, Completely Mixed Reactor. *Waste Management*. 30:1768–1771.

Gunaseelan, V.N. (1997). Anaerobic Digestion of Biomass for Methane Production: A Review. Biomass Bioenergy. 13: 83-114.

Hames, B.R..,Thomas, S.R., Sluiter, A.D., Roth, C.J.; and Templeton, D.W. (2003). Rapid Biomass Analysis - New Tools for Compositional Analysis of Corn Stover Feedstocks and Process Intermediates from Ethanol Production. *Applied Biochemistry and Biotechnology* (105:1-3); pp. 5-16.

Hanaki, K., Hirunmasuwan, S., and Matsuo, T. (1994). Protection of Methanogenic Bacteria From Low ph and Toxic Materials by Immobilization Using Polyvinyl Alcohol. *Water Research*. 28: 877-885.

Hartwig A, Klyszcz-Nasko H., Schlepegrell R., and Beyersmann D. (1993). Cellular damage by ferric nitrilitriacetate and ferric citrate in V79 cells: Interrelationship between lipid peroxidation, DNA strand breaks and sister chromatid exchange. *Carcinogenesis*, 14:107–112.

Hawkes, F.R. and Hawkes, D.L. (1987) *Anaerobic Digestion*, In Bu'lock, J. and B. Kristiansen (eds.), Basic Biotechnology. Academic press, London, 337-358.

Heeter, C. (2006). The Oil in Your Oatmeal: A Lot of Fossil Fuel Goes into Producing, Packaging and Shipping Our Breakfast. *San Francisco Chronicle*. P 6.

Hegde, G., and Pullammanappallil, P. (2007). Comparison of Thermophilic and Mesophilic One-Stage, Batch, High-Solids Anaerobic Digestion. *Environmental Technology*, 28: 361–369.

Hill, D.H. and Holmberg R. (1988). Long Chain Volatile Fatty Acid Relationships in Anaerobic Digester Failure. *Biological Wastes, 23*, 195-214.

Jain, S.R., and Mattiasson, B. (1998). Acclimatization of Methanogenic Consortia for low pH Biomethanation process. *Biotechnology Letters*, 20 (8), 771–775.

Jeongsik, K., Chulhwan, P., Tak-Hyun, K., Myunggu, L., Sangyong, K., Seung-Wook, K. and Jinwon, L. (2003). Effects of various Pretreatment for Enhanced

Anaerobic Digestion with waste Activated Sludge. *Journal of Bioscience and Bioengineering*, 95(3): 271-75.

Jha, A.K., He, J., Li, J. and Zheng, G. (2010). Effect of Substrate Concentration on Methane Fermentation of Cattle Dung. *In: Proceedings of International conference on challenges in environmental Science and computer engineering*. Wuhan, P. R. China. March 6-7. Part, 1: 512-515.

Jha, A.K., Pradhan, S., Li ,J., Hoover, M. (2010). Utilization of Municipal Solid Waste for Renewable Energy (Biogas and Heat-electricity) Production. *In: Proceedings of The Fifth International Conference on Environmental Science and Technology*. Houston, Texas, USA. July, 12-16.

Jones, R. W. (1994). *The Challenger Foraminifera.* Oxford University Press, Oxford,1-49.

Juanga, J.P. (2005). *Optimizing Dry Anaerobic Digestion of Organic Fraction of Municipal Solid Waste*. M. E. Thesis. Asian Institute of Technology, Bankok, Thailand, p 3.

Kanwar, S.S. and Guleri, R.L. (1994). Effect of recycling of digested slurry on biogas production. *Biogas Forum*, 4(59): 12–13.

Kapp, H. (1984). Schlammfaulung mit hohem Feststoffgehalt. Stuttgarter Berichte zur Siedlungswasserwirtschaft, Band 86, Oldenbourg Verlag, München, p. 300.

Karagiannidis A. and Perkoulidis G (2009). A Multi-Criteria Ranking of Different Technologies for the Anaerobic Digestion for Energy Recovery of the Organic Fraction of Municipal Solid Wastes. *Bioresource Technology.*100:2355–2360.

Kayhanian, M. (1999). Ammonia Inhibition in High-solids biogasification: an Overview and Practical Solutions. *Environmental Technology*. 20: 355–365.

Kenneth e. M., Curtis O. R., Robert N. M. and David W. D. (2001). H_2S Removal And Sulfur Recovery Technologies for CO_2 Streams; Seventh Annual CO_2 Conference*, University of Texas of the Permian Basin, Texas*, 12:4 – 5.

Klaassen A. J. (2006). *Nonparametric and Semiparametric Models* 10.1111/j.15410420.2006.00589_6.

Kobayashi F, S. T., Nakamura,Y., Mtui G.Y.S., Ushiyama, T. Potato wastes. (1998).Saccharification and Alcohol Fermentation in Starch Solution of Steam exploded, *Applied Biochemical Bioengineering*. 69: 43-55.

Kopp, J., Müller, J., Dichtl, N. and Schwedes, J. (1997). Anaerobic Digestion and Dewatering Characteristic of Mechanically Disintegrated Excess Sludge. *Water Science and Technology*, 36 (11),pp. 129–136.

Kottner, M. (2005). Biogas and Fertilizer Production from Solid Waste and Biomass Through Dry Fermentation in Batch Method, *International Biogas and Bioenergy Centre of Competence,* Germany, p 8.

Kroll, H. (1952). Metal Complexation Catalysis. *Journal of American Chemical Society,*74,2036.

Lastella, G., Testa, C., Cornacchia, G., Notornicola, M., Voltasio, F. and Sharma, V.K. (2002.) Anaerobic Digestion of Semi-Solid Organic Waste: Biogas Production and Its Purification. *Energy Conservation and Management*, 43,pp. 63-75.

Lay, J.J, Li, Y.Y., Noike, T., Endo, J.and Ishimoto, S. (1997). Analysis of Environmental Factors Affecting Methane Production from High-Solids Organic Waste. *Water Science and Technology*. 36(6-7): 493-500.

Lettinga,, G.(2001) Digestion and Degradation, Air for Life. *Water Science and Technology*, **44**(8).pp.157-176.

LfU (2007). Biogashandbuch Bayern - Materialband. - Bayerisches Landesamt für Umwelt, Augsburg, Germany.

Li, Y., Park, S.Y., and Zhu, J. (2011). Solid-State Anaerobic Digestion for Methane Production from Organic Waste. *Renewable and Sustainable Energy Reviews*. 15(1): 821-826.

Lissens, G., Vandevivere, P., de Baere, L., Biey, E.M. and Verstraete, W. (2001) Solid Waste Digesters: Process Performance and Practice for Municipal Solid Waste Digestion. *Water Science and Technology*, **44**, 91-102.

Lou. X.F., Nair, J. (2009). The Impact of Landfilling and Composting on Greenhouse Gas Emissions – A Review. *Bioresource Technology*.100(16): 3792-3798.

Lu, S., Imait, S., Ukita, M and Sekine, M. (2007). Start-up Performances of Dry Anaerobic Mesophilic and Thermophilic Digestions of Organic Solid Wastes. *Journal of Environmental Sciences.* 19: 416–420.

Malik, R.K., and Dahiya, D.S. (1990). Biogas Production from Cattle Waste by Recycling of Filtered Liquid of Digested Slurry. *Urja* 28, 30.

Malik, R.K., and Tauro, P. (1995). Effect of Predigestion and Effluent Slurry Recycling on Biogas Production. *Indian Journal Microbiology*.35 (3), 205–209.

Matthew L. W. (2013). New Solar Process Gets More Out of Natural Gas. *The New York Times*. P 3.

Marvin K. (1989). Release of Outer Membrane Fragment, *Journal of Bacteriology*, pp. 5262 – 5267.

Mital, K. (1996). Biogas Systems-Principles and Applications. *New age International* (P) Ltd, p 12.

Mohanrao, G.J., (1974). Scientific Aspects of Cow-dung Digestion. *Khadi Gramodyog*; 29 (7), 340–347.

Molnar, L. and Bartha I. (1988). High Solids Anaerobic Fermentation for Biogas and Compost Production. *Biomass London*. 16(3): 173-182.

Mosey F. E., Swanwick J. D. and Hughes D. A. (1971). Factors Affecting the Availability of Heavy Metals to inhibit Anaerobic Digestion. *Journal of Institute of Water Pollution Control 6* p 34-40.

Mosey, F.E. and Fernandes, X.A. (1989). Patterns of Hydrogen in Biogas from The Anaerobic-Digestion Of Milk-Sugars. *Water Science and Technology*. 21: 187–196.

Müller, J.A. (2001). Prospects and Problems of Sludge Pre-Treatment Processes. *Water Science and Technology* 44(10):121–128.

Müller, J.A., Winter, A. and Strünkmann, G. (2004). Investigation and Assessment of Sludge Pre-Treatment Processes. *Water Science and Technology*. 49(10):97–104.

Murray, D. (2006) Oil and Food: A Rising Security Challenge. *San Francisco Chronicle*, P 9.

Novak, J.T. (2007). Application of Mechanical Shear in an Internal Recycle for Enhancement of Mesophilic Anaerobic Digestion *Water Environment Research* 79(3):297-304.

Naomichi, N. and Yutaka, N. (2007). Recent Development of Anaerobic Muller, C.D., Abu-Orf, M. Digestion Processes for Energy Recovery From Wastes. *Journal of Bioscience and Bioengineering*. 103(2): 105–112.

Nges, I.A. and Liu, J .(2010). Effects of Solid Retention Time on Anaerobic Digestion of Dewatered-Sewage Sludge in Mesophilic and Thermophilic Conditions. *Renewable Energy*. 35(10): 2200-2206.

Oliveira, L.B, Rosa, L.P. (2003). Brazilian Waste Potential: Energy, Environmental, Social and Economic Benefits. *Energy Policy*. 31:1481-1491.

Ouedraogo, A. (1999). Pilot-scale Two-Phase Anaerobic Digestion of the Biodegradable Organic Fraction of Bamako District Municipal Solid Waste. In: *II International Symposium Anaerobic Digestion of Solid Waste*, Barcelona, June 15-17. (eds J. Mata-Alvarez, A.Tilche and F. Cecchi), Vol. 2, pp.73-76.

Patel, V., Patel, A.and Madamwar, D. (1992). Effect of Adsorbents on Anaerobic Digestion of Water-Hyacinth-Cattle Dung. *Bioresource Technology*.40 (2), 179–181.

Pearson, D. (1976). The Chemical Analysis of Foods, 7th(Ed.) Churchill Living stone, London, pp 10-11.

Pind, P. F., Angelidaki, I., Ahring, B. K., Stamatelatou, K. and Lyberatos, G. (2003c) Monitoring and control of anaerobic reactors. p.135-182, In: Ahring, B. K. (ed.) Biomethanation II. Springer, Berlin, pp 12-15.

Pinheiro J.P and van Leeuwen H.P,(2001) Speciation dynamics and bioavailability of metals. Exploration of the case of two uptake routes", *Pure and Applied Chemistry*, 73(1), pp. 39-44

Pohland, F.G. and Ghosh, S. (1971) Developments in Anaerobic Treatment Process. *Biotechnology and Bioengineering*, **2**, 85-106.

Prasad, K., Inmaculada, B., Lars, E, and Irini, A. (2008). Effects of Mixing on Methane Production During Thermophilic Anaerobic Digestion of Manure: Lab-Scale and Pilot-Scale Studies. *Bioresource Technology*. 99:4919–4928.

Rinzema, A., and Lettinga, G. (1988). The Effect of Sulphide on the Anaerobic Degradation of propionate. *Environmental Technology Letters* 9, 83–88.

Rittman, B.E., and McCarty, P.L. (2001). *Environmental Biotechnology: Principles and Applications. McGraw-Hill.* New York.

Saito, M. A., MoffetT, J.W., Chisholm, S.W. and Waterbury, J.B. (2002). Cobalt limitation and uptake in *Prochlorococcus. Limnology and Oceanography*. 47: 1629–1636.

Sandberg, M. and Ahring, B.K. (1992). Anaerobic Treatment of Fish-Meal Process Waste-Water in a UASB Reactor At High pH. *Applied Microbiology and Biotechnology*. 36: 800-804.

Santosh,V. P.and Chanel, S. (1999). Studies on Developing a Model for Water Conservation and Biogas System. Project Sponsored by DST (1996-99), CRDT, IIT, Delhi, India, pp11-19.

Schafer ,W., Lehto, M.and Teye, F .(2006). Dry Anaerobic Digestion of Organic Residues on-Farm-a Feasibility Study. *MTT Agrifood Research Reports* 77. Agricultural Engineering, Finland .<http://www.mtt.fi/met/pdf/met77.pdf>.

Schulte, D.D. and Kottwitz, D. (1982). *Bioconversion of Animal Manure to Methane Gas Using an Innovative Plug-Flow Digester*. Final Report. In DOE/R7/01042-T1, Nebraska Univ., Lincoln (USA). Dept. Of Agricultural Engineering.

Sharma, S.K., Mishra, I.M., Sharma, M.P. and Saini, J.S. (1988). Effect of Particle Size on Biogas Generation from Biomass Residues. *Biomass* 17, 251–263.

Song, Z., Yang, G., Guo Y., and Zhang, T. (2012) Comparison of two Chemical Pretreatments. of Rice Straw for Biogas Production by Anaerobic Digestion. *Biological Resources.* 7: 3223–3236.

Stewart, D.J., Bogue, M.J. and Badger, D.M. (1984). Biogas Production from Crops and Organic Wastes 2. Results of Continuous Digestion Tests. *New Zealand Journal of Science*, 27, 285-294.

Styring, S. (2011). Artificial photosynthesis for solar fuels. *Faraday Discussions* (Advance Article). doi:10.1039/C1FD00113B.

Takizawa, N., Umetsu, K., Takahata, H., Hoshiba, H. (1994).Temperature Effects on Continuously Expending Anaerobic Digester with Dairy Manure Slurry. *Resource Bulletin Obihiro University*, *Natural Science*. 19(1), 31–36.

Teodorita, A.S., Dominik, R.., Heinz, P., Michael, K., Tobias, F., Silke, V. and Rainer J. (2008). *Biogas Handbook*. University of Southern Denmark, Esbjerg, Niels Bohrs, Denmark, pp. 10 – 50.

Tirumale, S.and Nand. K .(1994). Influence of Anaerobic Cellulolytic Bacterial Consortia in the Anaerobic Digesters on Biogas Production. *Biogas Forum III* (58), 12–15.

Uemura, S.H. (2010). Mineral Requirements for Mesophitic and thermophitic Anaerobic Digestion of Organic waste. International Journal of Enviromental Research, 4(1): 33-4.

Umetsu, K., Takahata, H. and Kawamoto, T. (1992). Effect of Temperature on Mesophilic Anaerobic Digestion of Dairy Cow Slurry. *Resource Bulletin of Obihiro University.Services.I* 17 (4), 401–408.

Vavilin,V.A., Rytov, S.V., Lokshina, L.Y., Rintala, J. A. and Lyberatos, G.(2001) Simplified Hydrolysis Models for the Optimal Design of Two-Stage Anaerobic Digestion. *Water Research*, 35, 4247- 4251.

Viéitez, E.R, Mosquera, J., and Ghosh, S. (2000). Kinetics of Accelerated Solidstate Fermentation of Organic-Rich Municipal Solid Waste. *Water Science and Technology*. 41(3): 231-238.

Wang, Q., Kuninobu, M., Kakimoto, K., Ogawa, H.I. and Kato, T.,(1999). Upgrading of Anaerobic Digestion of Waste Activated Sludge by Ultrasonic Pretreatment. *Bioresource Technology*. 68 (3), 309–313.

Wang, X., Yang, G., Feng, Y., Ren, G., and Han, X. (2012). Optimizing Feeding Composition and Carbon-Nitrogen ratios for Improved Methane yield during Anaerobic Co-digestion of Dairy, Chicken Manure and Wheat Straw. *Bioresoures Technology* 120: 78–83.

Ward, A.J, Honns, P.J., Holliman, P.J., and Jonesmann, D.J. (2008). Optimisation of the Anaerobic Digestion of Agricultural Resources. *Bioresource Technology*., 99 (17): 7928-7940.

Wawrzynczyk, J, Dey, E., Norrlöw, O. and Jansen, J.C. (2003). Alternative Method for Sludge Reduction using Commercial Enzymes. *In: Proceedings of the 8th European Biosolids and Organic Residuals Conference*. Wakefield, West Yorkshire, UK,pp.1 – 5.

Weiland, P. (2010) Biogas Production: current state and perspectives. *Applied Microbiology Biotechnology*. 85: 849–860.

Winfrey, M.R., and Zeikus, J.G.,(1977).Effect of Sulfate on Carbon and Electron Flow During Microbial Methanogenesis in Freshwater Sediments. *Applied Environmental Microbiology*. 33, 275–281.

Willy, Y.W. (2010). Biogas Fermentation and Microbial Fuel cell. *Journal of Science Engineering*.20, 3-4.

Worm, B., E. B. Barbier, N. Beaumont, J.E. Duffy, C. Folke, B.S. Halpern, J.B.C. Jackson, H.K. Lotze, F. Micheli, S.R. Palumbi, E. Sala, K.A. Selkoe, J.J. Stachowicz and R. Watson. (2006). Impacts of Biodiversity Loss on Ocean Ecosystem Services. *Science* 314:787-790.

Zhu, D. (2010). Co-digestion of Different Wastes for Enhanced Methane Production, M.Sc Thesis, The Ohio State University, p5.

APPENDIX

APPENDIX I: Results Showing the Percentage of CH_4 gas yield from the Various Weights used

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
150gGU	50	1.90	8.30	2.3740	0.15415	1.08999
200gGU	50	2.00	4.90	2.2640	0.06832	0.48309
250gGU	50	1.90	2.60	2.1460	0.02123	0.15012
150gCM	50	2.00	51.90	18.5900	1.94368	13.74392
200gCM	50	2.00	61.30	24.2400	2.25448	15.94156
250gCM	50	1.90	75.40	30.1960	2.77888	19.64963
150gCH	50	2.00	84.80	21.4280	3.58735	25.36639
200gCH	50	2.00	23.30	6.2880	0.91373	6.46102
250gCH	50	1.90	48.90	5.2100	1.26350	8.93428
150gCD	50	2.00	67.60	20.0560	2.98422	21.10162
200gCD	50	2.00	53.80	14.6900	2.20948	15.62336
250gCD	50	2.00	51.10	11.2520	1.90883	13.49750
150gBL	50	2.00	33.30	5.9440	0.80889	5.71972
200gBL	50	2.00	20.00	4.5420	0.58800	4.15781
250gBL	50	2.00	13.80	3.0400	0.27590	1.95093
Valid N (listwise)	50					

APPENDIX II: Results Showing the Effect of Addition of Chelating Ligand on CH_4 gas Yield

CM Substrate

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
EDDACM	25	5.50	70.50	37.6440	4.40636	22.03178
MESYLCM	25	17.50	64.20	42.7120	2.95684	14.78418
NTACM	25	14.50	72.20	47.1600	3.36268	16.81339
PENICM	25	5.60	58.50	22.1240	2.80097	14.00486
EDTACM	25	11.00	59.00	28.3160	2.83672	14.18358
DTPACM	25	5.60	65.60	33.2640	3.68539	18.42693
PORPHCM	25	4.60	59.60	25.6720	2.89052	14.45258
250gCM	25	1.90	64.40	22.9800	4.40840	22.04199
Valid N (listwise)	25					

CD Substrate

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
EDDA CD 10µM	30	3.70	53.10	26.4133	2.71344	14.86212
MESYL CD 10µM	30	7.00	64.60	34.0200	3.02804	16.58525
NTA CD 10µM	30	9.50	59.90	32.9767	2.52314	13.81982
DTPA CD 10µM	30	2.40	66.90	36.4567	3.75346	20.55852
EDTA CD 10µM	30	4.10	70.30	38.1333	3.57062	19.55708
PENI CD 10µM	30	6.50	65.70	40.5400	3.69319	20.22843
PORPH CD 10µM	30	4.50	67.40	34.4267	3.60156	19.72654
150gCD	30	2.00	67.60	12.6667	3.60602	19.75097
Valid N (listwise)	30					

CH Substrate

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
EDDA CH 10µM	32	0.70	57.20	17.6563	2.92600	16.55193
MESYL CH 10µM	32	1.70	69.40	15.5188	3.16511	17.90454
DTPA CH 10µM	32	1.70	24.50	5.2563	0.74643	4.22244
PENI CH 10µM	32	1.60	9.60	2.9375	0.30033	1.69891
EDTA CH 10µM	32	1.50	15.90	4.1219	0.63358	3.58408
NTA CH 10µM	32	1.30	22.00	3.8531	0.72554	4.10428
PORPH CH 10µM	32	1.50	21.60	5.0125	0.79533	4.49909
150gCH	32	2.00	13.60	5.8656	0.69531	3.93328
Valid N (listwise)	32					

APPENDIX III: Results Showing the Effect of Increasing Chelating Ligand Concentration on CH_4 gas Yield

CM Substrate

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
250gCM	50	1.90	75.40	30.1960	2.77888	19.64963
EDDA CM 10µM	25	5.50	70.50	37.6440	4.40636	22.03178
EDDA CM 50µM	19	2.40	52.10	18.6947	3.41588	14.88946
EDDA CM 100µM	19	4.10	43.40	17.6947	2.58946	11.28718
Valid N (listwise)	19					

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
250gCM	50	1.90	75.40	30.1960	2.77888	19.64963
NTA CM 10µM	25	14.50	72.20	47.1600	3.36268	16.81339
NTA CM 50µM	19	2.00	43.70	22.6368	3.48959	15.21075
NTA CM 100µM	19	1.90	45.10	17.1842	3.29003	14.34090
Valid N (listwise)	19					

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
250gCM DTPA CM 10µM	50	1.90	75.40	30.1960	2.77888	19.64963
250gCM DTPA CM 50µM	25	5.40	65.60	33.2560	3.68789	18.43947
250gCM DTPA CM 100µM	19	1.00	46.90	16.3105	3.29652	14.36918
Valid N (listwise)	19	2.00	48.60	18.5789	3.15741	13.76282

CD Substrate

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
150gCD EDTA CD 10µM	50	2.00	67.60	20.0560	2.98422	21.10162
150gCD EDTA CD 50µM	30	4.10	70.30	38.1333	3.57062	19.55708
150gCD EDTA CD 100µM	25	3.30	43.70	14.7360	2.26644	11.33218
Valid N (listwise)	25	5.80	60.50	19.3440	2.87598	14.37988

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
150gCD	50	2.00	67.60	20.0560	2.98422	21.10162
PORPH CD 10µM	30	4.50	67.40	34.4267	3.60156	19.72654
PORPH CD 50µM	25	5.50	65.90	17.5040	2.68160	13.40800
PORPH CD 100µM	25	4.10	59.50	13.6000	2.42568	12.12838
Valid N (listwise)	25					

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
150gCD	50	2.00	67.60	20.0560	2.98422	21.10162
DTPA CD 10µM	30	2.40	66.90	36.4567	3.75346	20.55852
DTPA CD 50µM	25	3.90	55.90	15.6720	2.55583	12.77913
DTPA CD 100µM	25	2.50	54.20	15.5640	2.47746	12.38729
Valid N (listwise)	25					

CH Substrate

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
150gCH	50	2.00	84.80	21.4280	3.58735	25.36639
EDDA CH 10µM	32	0.70	57.20	17.6563	2.92600	16.55193
EDDA CH 50µM	29	1.10	64.30	24.1517	4.41838	23.79369
EDDA CH 100µM	30	1.00	63.10	25.2500	4.30035	23.55401
Valid N (listwise)	29					

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
150gCH	50	2.00	84.80	21.4280	3.58735	25.36639
MESYL CH 10µM	32	1.70	69.40	15.5188	3.16511	17.90454
MESYL CH 50µM	29	1.00	71.10	24.4483	4.81905	25.95139
MESYL CH 100µM	30	0.90	19.80	7.5433	1.14060	6.24730
Valid N (listwise)	29					

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
150gCH	50	2.00	84.80	21.4280	3.58735	25.36639
DTPA CH 10µM	32	1.70	24.50	5.2563	0.74643	4.22244
DTPA CH 50µM	29	0.90	14.00	5.6793	0.75514	4.06653
DTPA CH 100µM	30	1.00	75.30	23.5200	4.87789	26.71730
Valid N (listwise)	29					

APPENDIX IV: Result Showing the Effect of Addition of Chelating Ligand on VFA Concentration

CM Substrate

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CM VFA	3	644.48	4431.21	2009.1633	1214.26850	2103.17473
EDDA CM	3	476.05	4422.95	2044.9767	1209.05229	2094.13999
NTA CM	3	671.96	4688.28	2215.4800	1249.04998	2163.41803
EDTA CM	3	1451.20	4810.38	2953.0300	985.86991	1707.57677
MESYL CM	3	949.49	4568.49	2289.4367	1145.38658	1983.86775
DTPA CM	3	737.63	5392.17	2848.5667	1360.95383	2357.24118
PORPH CM	3	1058.83	4489.73	3205.4800	1080.21125	1870.98076
PENI CM	3	686.92	5838.00	3173.1767	1489.66672	2580.17845
Valid N (listwise)	3					

CD Substrate

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CD VFA	3	493.65	1177.52	789.8433	202.64620	350.99351
EDDA CD	3	65.75	1161.13	489.1767	339.74915	588.46280
NTA CD	3	628.51	843.56	709.1033	67.66971	117.20738
EDTA CD	3	483.75	1585.40	976.1833	323.33519	560.03298
MESYL CD	3	267.82	1098.54	620.8467	247.77696	429.16228
DTPA CD	3	419.73	1512.03	846.3833	337.20329	584.05324
PORPH CD	3	127.24	1733.68	793.3567	483.58189	837.58841
PENI CD	3	311.38	1388.39	1005.7733	347.79839	602.40448
Valid N (listwise)	3					

CH Substrate

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CH VFA	3	3209.55	6618.34	5061.1800	994.98689	1723.36785
EDDA CH	3	9204.55	14364.90	12242.2833	1558.35190	2699.14466
DTPA CH	3	9665.78	28112.01	17908.8867	5414.39745	9378.01148
EDTA CH	3	10628.72	40036.56	24458.3233	8534.21647	14781.69653
MESYL CH	3	9277.92	15939.96	13135.8400	1994.03820	3453.77548
NTA CH	3	10239.32	39069.44	23480.7900	8404.87746	14557.67479
PORPH CH	3	9755.54	42414.98	21827.9300	10344.63358	17917.43094
PENI CH	3	9269.67	40457.75	24017.2600	9042.92571	15662.80678
Valid N (listwise)	3					

APPENDIX V: Result Showing the Effect of Increasing Chelating Ligand Concentration on VFA

CM Substrate

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CM VFA	3	728.61	4431.21	2037.2067	1198.73439	2076.26887
EDDA CM 50µM	3	2112.19	8929.55	6336.9600	2130.50135	3690.13658
NTA CM 50µM	3	561.30	3782.21	1943.4500	957.41576	1658.29274
EDTA CM 50µM	3	1013.84	3517.99	2171.7733	728.99012	1262.64792
EDDA CM 100µM	3	1719.71	7002.57	5237.5100	1758.90360	3046.51039
NTA CM 100µM	3	518.78	4304.49	1983.8367	1173.58912	2032.71599
EDTA CM 100µM	3	1010.21	3782.21	2033.5767	878.54137	1521.67829
Valid N (listwise)	3					

CD Substrate

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CD VFA	3	493.65	1177.52	789.8433	202.64620	350.99351
EDDA CD 50µM	3	1412.48	1553.06	1487.3900	40.84408	70.74403
DTPA CD 50µM	3	1000.53	1759.31	1403.0933	220.26331	381.50725
PORPH CD 50µM	3	206.33	1895.27	1229.0000	519.10028	899.10806
EDDA CD 100µM	3	863.03	1892.19	1317.0733	303.19776	525.15392
DTPA CD 100µM	3	995.91	1773.17	1386.8500	224.38753	388.65060
PORPH CD 100µM	3	675.26	1377.61	1038.6633	203.11940	351.81313
Valid N (listwise)	3					

CH substrate

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CH VFA	3	3209.55	6618.34	5061.1800	994.98689	1723.36785
EDDA CH 50µM	3	12042.11	12924.53	12361.7333	282.26242	488.89285
DTPA CH 50µM	3	7584.80	11946.19	10271.9533	1357.07170	2350.51714
MESYL CH 50µM	3	11077.08	11552.17	11255.4633	149.36302	258.70434
EDDA CH 100µM	3	7027.10	12152.44	9905.4700	1512.86450	2620.35818
DTPA CH 100µM	3	11048.37	14112.75	12249.9000	944.38945	1635.73051
MESYL CH 100µM	3	9141.41	11233.06	10094.6333	610.86691	1058.05252
Valid N (listwise)	3					

APPENDIX VI: Result Showing H_2S Concentration in the various Substrates

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
GU H_2S	50	1.00	5000.00	405.0800	173.90506	1229.69450
CM H_2S	50	1.00	971.00	73.8000	24.50891	173.30414
CH H_2S	50	1.00	5000.00	614.1900	165.46739	1170.03116
CD H_2S	50	1.00	1014.00	149.3800	38.29124	270.75992
BL H_2S	50	1.00	2944.00	171.0400	57.50512	406.62257
Valid N (listwise)	50					

APPENDIX VII: Results Showing the Effect of Increasing Chelating Ligand Concentration on Percentage of H_2S

CM Substrate

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CM H_2S	50	1.00	971.00	73.8000	24.50891	173.30414
DTPA CM 10µM	26	1.00	465.00	79.1154	22.41691	114.30427
DTPA CM 50µM	19	1.00	30.00	10.1579	2.13062	9.28717
DTPA CM 100µM	19	1.00	122.00	26.2632	8.74721	38.12821
Valid N (listwise)	19					

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CM H_2S	50	1.00	971.00	73.8000	24.50891	173.30414
EDDA CM 10µM	26	0.00	270.00	33.9231	12.00883	61.23327
EDDA CM 50µM	19	1.00	138.00	44.6842	8.70473	37.94302
EDDA CM 100µM	19	5.00	148.00	32.5789	8.47298	36.93285
Valid N (listwise)	19					

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CM H_2S	50	1.00	971.00	73.8000	24.50891	173.30414
NTA CM 10µM	26	1.00	404.00	47.0769	17.44662	88.96063
NTA CM 50µM	19	1.00	216.00	31.3684	11.98713	52.25069
NTA CM 100µM	19	1.00	144.00	20.5789	9.43160	41.11139
Valid N (listwise)	19					

CD Substrate

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CD H_2S	52	1.00	1014.00	143.8462	37.00743	266.86439
EDTA CD 10µM	30	0.00	344.00	63.3333	15.91972	87.19591
EDTA CD 50µM	25	0.00	526.00	121.2800	29.05544	145.27718
EDTA CD 100µM	25	0.00	364.00	108.5200	22.55505	112.77526
Valid N (listwise)	25					

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CD H_2S	52	1.00	1014.00	143.8462	37.00743	266.86439
DTPA CD 10µM	30	0.00	185.00	35.8000	8.29200	45.41715
DTPA CD 50µM	25	4.00	289.00	95.2400	15.68240	78.41199
DTPA CD 100µM	25	3.00	267.00	67.6400	15.93611	79.68055
Valid N (listwise)	25					

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CD H_2S	52	1.00	1014.00	143.8462	37.00743	266.86439
PORPH CD 10µM	30	1.00	161.00	42.3000	8.50870	46.60409
PORPH CD 50µM	25	2.00	227.00	93.4800	13.56615	67.83075
PORPH CD 100µM	25	0.00	206.00	46.0000	11.75287	58.76436
Valid N (listwise)	25					

CH substrate

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CH H_2S	50	1.00	5000.00	614.1900	165.46739	1170.03116
EDDA CH 10µM	32	1.00	979.00	190.0313	42.98923	243.18379
EDDA CH 50µM	29	3.00	1872.00	311.2069	80.65766	434.35481
EDDA CH 100µM	30	3.00	1594.00	286.7667	80.59070	441.41346
Valid N (listwise)	29					

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CH H_2S	50	1.00	5000.00	614.1900	165.46739	1170.03116
DTPA CH 10µM	32	1.00	5000.00	580.0000	182.55788	1032.70334
DTPA CH 50µM	29	0.00	1433.00	202.8621	64.25118	346.00317
DTPA CH 100µM	30	0.00	1162.00	197.5333	48.38989	265.04232
Valid N (listwise)	29					

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CH H_2S	50	1.00	5000.00	614.1900	165.46739	1170.03116
MESYL CH 10µM	32	6.00	3429.00	302.9375	108.69796	614.88850
MESYL CH 50µM	29	0.00	729.00	168.6552	32.52582	175.15692
MESYL CH 100µM	30	5.00	1051.00	153.1667	41.53030	227.47081
Valid N (listwise)	29					

APPENDIX VIII: Results Showing the Percentage of CH_4 produced from the Different Blends in Co-digestion

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CH CM	35	3.20	78.80	37.4943	4.16076	24.61539
CH GU	35	1.80	23.30	11.1143	1.02169	6.04440
GU BL	35	1.10	3.90	2.0314	0.12111	0.71652
CH BL	35	1.20	6.30	2.4857	0.22403	1.32535
CM BL	35	1.70	8.70	4.0029	0.35500	2.10021
CM GU	35	1.70	14.20	4.2743	0.43247	2.55853
Valid N (listwise)	35					

APPENDIX IX: Results Showing the Effect of Addition of Chelating Ligand on CH:CM Co-digestion

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CH CM	35	3.20	78.80	37.4943	4.16076	24.61539
EDDA CHCM 10µM	26	1.40	79.60	26.2769	4.80947	24.52359
NTA CHCM 10µM	26	2.20	68.20	22.7846	4.70888	24.01066
EDTA CHCM 10µM	26	2.80	70.10	20.2000	4.18015	21.31467
DTPA CHCM 10µM	26	1.90	73.60	21.3846	4.23041	21.57094
MESYL CHCM 10 µM	26	2.70	71.00	19.2615	3.98522	20.32073
PORPH CHCM 10µM	26	2.40	58.30	24.8846	3.58467	18.27831
PENI CHCM 10µM	26	1.50	57.60	20.0500	3.43245	17.50214
Valid N (listwise)	26					

APPENDIX X: Results Showing the Effect of Increasing Chelating Ligand Concentration on Co-digestion

EDDA Chelating Ligand

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CH CM	35	3.20	78.80	37.4943	4.16076	24.61539
EDDA CHCM 10µM	26	1.40	79.60	26.2769	4.80947	24.52359
EDDA CHCM 50µM	21	6.40	71.90	30.6571	4.26218	19.53178
EDDA CHCM 100µM	21	14.40	61.10	34.3762	3.68163	16.87136
Valid N (listwise)	21					

DTPA Chelating Ligand

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CH CM	35	3.20	78.80	37.4943	4.16076	24.61539
DTPA CHCM 10µM	26	1.90	73.60	21.3846	4.23041	21.57094
DTPA CHCM 50µM	21	10.60	69.00	35.5238	3.95095	18.10555
DTPA CHCM 100µM	21	9.40	59.40	29.8048	3.86835	17.72700
Valid N (listwise)	21					

MESYL Chelating Ligand

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CH CM	35	3.20	78.80	37.4943	4.16076	24.61539
MESYL CHCM 10 µM	26	2.70	71.00	19.2615	3.98522	20.32073
MESYL CHCM 50µM	21	12.00	69.00	35.0238	4.06204	18.61462
MESYL CHCM 100µM	21	12.10	70.40	33.3429	3.87714	17.76729
Valid N (listwise)	21					

APPENDIX XI: Result Showing the Effect of Addition of Chelating Ligand on VFA Concentration in Co-digestion

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CH:CM	3	1979.31	6659.37	3603.1300	1529.11856	2648.51103
EDDA CH:CM 10µM	3	4362.35	9827.37	7393.2900	1605.59359	2780.96967
NTA CH:CM 10µM	3	6831.19	9799.10	8105.0633	882.14235	1527.91536
EDTA CH:CM 10µM	3	2378.50	4318.35	3279.2533	564.24246	977.29661
DTPA CH:CM 10µM	2	5128.39	7775.21	6451.8000	1323.41000	1871.58437
MESYL CH:CM 10µM	3	6735.27	9324.56	8194.3467	765.33631	1325.60137
PORPH CH:CM 10µM	3	1529.41	3154.77	2481.6433	489.51479	847.86448
PENI CH:CM 10µM	3	4753.40	6042.16	5207.7733	417.74521	723.55593
Valid N (listwise)	2					

APPENDIX XII: Result Showing the Effect of Increasing Chelating Ligand Concentration on VFA

AT 50 µM

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CH:CM	3	1979.31	6659.37	3603.1300	1529.11856	2648.51103
EDDA CH:CM 50µM	3	2603.67	6909.18	4325.9767	1315.32143	2278.20355
NTA CH:CM 50µM	3	2930.98	6774.76	4255.8533	1260.01956	2182.41790
EDTA CH:CM 50µM	3	2653.94	6513.62	4225.0700	1170.51285	2027.38773
DTPA CH:CM 50µM	3	2605.21	6504.38	5062.0233	1234.60161	2138.39272
MESYL CH:CM 50µM	3	2677.04	6862.98	5437.2333	1380.34831	2390.83340
PORPH CH:CM 50µM	3	2414.36	5876.94	4009.4333	1008.79962	1747.29219
PENI CH:CM 50µM	3	2467.71	5354.66	3568.5533	901.07518	1560.70800
Valid N (listwise)	3					

AT 100 µM

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CH:CM	3	1979.31	6659.37	3603.1300	1529.11856	2648.51103
EDDA CH:CM 100µM	3	2808.38	8365.69	6089.3133	1681.04870	2911.66176
NTA CH:CM 100µM	3	2907.38	7144.14	5672.8900	1383.69861	2396.63630
EDTA CH:CM 100µM	3	2711.91	7460.17	5671.8633	1490.64418	2581.87145
DTPA CH:CM 100µM	3	2786.82	7542.78	4593.0967	1487.20353	2575.91207
MESYL CH:CM 100µM	3	2544.16	8243.59	5708.6400	1675.12254	2901.39735
PORPH CH:CM 100µM	3	2609.83	6257.10	4525.0400	1056.85107	1830.51975
PENI CH:CM 100µM	3	2925.86	6840.43	4305.6267	1269.06092	2198.07799
Valid N (listwise)	3					

APPENDIX XIII: Result Showing Effect of Chelating Ligand on H_2S Concentration in Co-digestion

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CHCM H_2S	33	2.00	862.00	99.6364	28.48760	163.64879
EDDA H_2S CHCM 10µM	26	0.00	5000.00	615.2308	269.61563	1374.77534
EDDA CHCM H_2S 50µM	21	1.00	853.00	188.0000	53.58758	245.56913
EDDA CHCM H_2S 100µM	21	1.00	706.00	133.5714	45.75184	209.66129
Valid N (listwise)	21					

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CHCM H_2S	33	2.00	862.00	99.6364	28.48760	163.64879
DTPA H_2S CHCM 10µM	26	0.00	3425.00	198.0769	133.87724	682.64268
DTPA CHCM H_2S 5OµM	21	6.00	756.00	136.4286	44.77657	205.19200
DTPA CHCM H_2S 100µM	21	3.00	481.00	119.5238	32.62176	149.49168
Valid N (listwise)	21					

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
CHCM H_2S	33	2.00	862.00	99.6364	28.48760	163.64879
MESYL H_2S CHCM 10µM	26	0.00	3526.00	262.3462	146.51648	747.09041
MESYL CHCM H_2S 50µM	21	3.00	769.00	171.6190	47.54229	217.86612
MESYL CH CM H_2S 100µM	21	2.00	372.00	115.4286	28.56851	130.91737
Valid N (listwise)	21					