RELATIONSHIP BETWEEN SILVER INHIBITION AND FEEDING OF ACTIVATED SLUDGE

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ABSTRACT

RELATIONSHIP BETWEEN SILVER INHIBITION AND FEEDING OF ACTIVATED SLUDGE

Silver is one of the heavy metals which is widely used in industrial, electronics and medical applications. Increase in the demand of silver and silver containing products cause accumulation of silver in sewer systems. Since silver ions are highly toxic on microorganisms, they affect biological wastewater treatment systems.

The objective of this study is to examine the effect of silver ion on different types of activated sludge. Activated sludge was fed with synthetic wastewaters which had different C/N (COD/Nitrogen) ratios and inhibitory effect of silver ion was examined by using a respirometer.

The result of the study shows that the C/N ratio in wastewater has a crucial role because it determines the relative ratio between heterotrophs and autotrophs in activated sludge. The inhibitory effect of silver ion changes with the C/N ratio in a wastewater. An activated sludge enriched in terms of autotrophs (C/N ratio = 0) is more sensitive to silver ion than other sludge types. On the other hand, with the increase in C/N ratio heterotrophs dominate in activated sludge and they can tolerate higher concentrations of silver ion.

ÖZET

AG AĞIR METALİNİN AKTİF ÇAMUR ÜZERİNDEKİ İNHİBİSYON ETKİSİ İLE ÇAMUR BESLENMESİ ARASINDAKİ İLİŞKİ

Gümüş, endüstride, elektronikte ve medikal uygulamalarda yaygın bir şekilde kullanılan ağır bir metaldir. Gümüş ve gümüş içeren ürünlerin kullanımı, kanalizasyon sistemlerinde gümüş birikmesine sebep olur. Gümüş iyonları mikroorganizmalar üzerinde toksik etkilere sahip olduğu için atıksu arıtma sistemlerini olumsuz etkiler.

Bu çalışmanın amacı, gümüş iyonlarının farklı tipteki aktif çamur sistemleri üzerine olan etkisini incelemektir. Aktif çamur farklı KOI/Azot (C/N) oranlarına sahip sentetik atıksular ile beslenmiş ve gümüş iyonlarının inhibisyon etkileri respirometre kullanılarak tetkik edilmiştir.

Çalışmanın sonuçları, atıksuyun C/N oranının aktif çamur içerisindeki heterotrof ve ototrofların birbirlerine olan oranını etkilediğini göstermiştir. Ayrıca, C/N oranının değişimiyle gümüş metalinin inhibisyon etkisinin değiştiği görülmüştür. Nitrifikasyon bakterilerince zengin aktif çamur (C/N oranı = 0) diğer çamur tiplerine göre daha hassas olup, gümüş metalinden daha çok etkilenmektedir. Diğer yandan, atıksudaki C/N oranının yükselmesiyle hetetotrofik aktivite önem kazandığından, aktif çamurun yüksek gümüş konsantrasyonlarını tolere edebildiği saptanmıştır.

TABLE OF CONTENTS

ACKNOWLEDGEMENT iii
ABSTRACT iv
ÖZET v
LIST OF FIGURES ix
LIST OF TABLES xiii
LIST OF SYMBOLS / ABBREVIATIONS xv
1. INTRODUCTION 1
2. LITERATURE REVIEW 3
2.1. Activated Sludge Process 3
2.1.1. Definition and Features of the Activated Sludge Process 3
2.1.2. Substrate Removal Mechanisms in Activated Sludge 6
2.1.2.1 Organic Carbon Removal. 6
2.1.2.2. Nitrification. 11
2.1.3. Physical and Chemical Characterization of Activated Sludge 14
2.1.4. Factors Affecting the Properties of Activated Sludge 17
2.1.4.1. Impact of the C/N Ratio on the Characteristics of Activated Sludge. 18
2.1.4.2. Impact of the Carbon Source in a Wastewater on the Characteristics on Activated Sludge. 19
2.2. Inhibition of Activated Sludge 20
2.2.1. Inhibition Mechanisms 21
2.2.2. Inhibition of Organic Carbon Removal 23
2.2.3. Inhibition of Nitrification 26
2.2.3.1. Substrate Inhibition in Nitrification. 26
2.2.3.2. Inhibition of Nitrification by Inorganic and Organic Compounds. 28
2.2.3.3. Inhibition of Nitrification by Specific Inhibitors. 31
2.3. Inhibition of Activated Sludge by Silver 32
2.3.1. Properties and Sources of Silver Metal 32
2.3.2. Inhibitory Effect of Silver 36

2.4. Respirometry of Activated Sludge and Respiration Inhibition 39
3. MATERIALS AND METHODS 41
3.1. Materials 41
3.1.1. Synthetic Domestic Wastewater 41
3.1.2. Activated Sludge 44
3.1.3. Silver Used in Respirometry Tests 45
3.1.4. Nitrification Inhibitors 45
3.2. Analytical Methods 47
3.2.1. Chemical Oxygen Demand (COD) Analysis 47
3.2.2. NH_4-N Analysis 47
3.2.3. NO_2-N Analysis 48
3.2.4. NO_3-N Analysis 48
3.2.5. TKN Analysis 48
3.2.6. Alkalinity Analysis 49
3.2.7. pH Analysis 49
3.2.8. MLSS & MLVSS Analysis 49
3.3. Experimental Work 50
3.3.1. Monitoring of Activated Sludge Reactors 50
3.3.2. Respirometry Tests 50
3.3.2.1. Tests for Determination of the Sensitivity of Respirometric Data. 53
3.3.2.2. Tests for Determination of Organic Carbon Removal and Nitrification. 53
3.3.2.3. Respiration Inhibition Tests with Ag. 54
3.3.2.4. Evaluation of Respirometry Data. 54
4. RESULTS AND DISCUSSION 58
4.1. Operation of Activated Sludge Reactors 58
4.1.1. Removal of Organic Carbon in Reactors 59
4.1.2. Removal of Nitrogen in Reactors 61
4.1.3. pH Profiles of Reactors 64
4.1.4. MLSS and MLVSS Profiles in Reactors 66
4.2. Respirometry Tests 69
4.2.1. Determination of the Sensitivity of Respirometric Data 73
4.2.2. Determination of Organic Carbon Removal and Nitrification 77

4.2.2.1. Results of Reactor 1 (C/N Ratio: 10). 77
4.2.2.2. Results of Reactor 2 (C/N Ratio: 5). 83
4.2.2.3. Results of Reactor 3 (C/N Ratio of 0). 88
4.2.3. Determination of the Effect of Ag on Activated Sludge 89
4.2.3.1. Results of Reactor 1. 90
4.2.3.2. Results of Reactor 2. 96
4.2.3.3. Results of Reactor 3. 100
5. CONCLUSIONS AND RECOMMENDATIONS 102
REFERENCES 104
APPENDIX A: MONITORING OF ACTIVATED SLUDGE REACTORS 114
APPENDIX B: RAW DATA IN RESPIROMETRIC TESTS 141

LIST OF FIGURES

Figure 2.1. Scheme of typical activated sludge process 4
Figure 2.2. Bacterial metabolisms 6
Figure 2.3. COD fractionation of wastewater 8
Figure 2.4. Substrate utilization for energy production and growth 9
Figure 2.5. Nitrogenous compounds and N conversation pathways in activated sludge systems 13
Figure 2.6. Schematic presentation of silver flow from industrial applications to the environment. 35
Figure 2.7. Global silver cycle 35
Figure 2.8. Antimicrobial effects of Ag^{+} 36
Figure 3.1. Configuration of reactors (R1, R2, R3 and CR) 45
Figure 3.2. Block diagram of gas flow through ER-10 Respirometer 51
Figure 3.3. Configuration of respirometry tests 53
Figure 3.4. Example of N-O_2 calculation from raw respirometric data 56
Figure 3.5. Example of graphical presentation of N-O_2 57
Figure 4.1. MLSS and MLVSS results in the main reactor 58
Figure 4.2. COD profiles in Reactor 1 (R1) 59
Figure 4.3. COD profiles in Control Reactor (CR) 60
Figure 4.4. COD Profiles in Reactor 2 (R2) 60
Figure 4.5. Influent and effluent NH_4-N values in Reactor 1 (R1) 61
Figure 4.6. Influent and effluent NH_4-N values in Control Reactor (CR) 62
Figure 4.7. Influent and effluent NH_4-N values in Reactor 2 (R2) 62
Figure 4.8. Influent and effluent NH_4-N in Reactor 3 (R3) 63
Figure 4.9. Alkalinity values in the influent and effluent of Reactor 3 (R3) 64
Figure 4.10. pH values under initial and final conditions of Reactor 1 (R1) 65
Figure 4.11. pH values under initial and final conditions of Reactor 2 (R2) 65
Figure 4.12. pH values under initial and final conditions of Reactor 3 (R3) 66
Figure 4.13. pH values under initial and final conditions of Control Reactor (CR) 66
Figure 4.14. MLSS and MLVSS values in Reactor 1 (R1) 67

Figure 4.15. MLSS and MLVSS values in Reactor 2 (R2) 67
Figure 4.16. MLSS and MLVSS values in Reactor 3 (R3) 68
Figure 4.17. MLSS and MLVSS values of Control Reactor (CR) 68
Figure 4.18. Relationship between oxygen utilization and biomass growth 72
Figure 4.19. Average values and 95 % confidence interval of respirometric data in Reactor 1 (Test 20) 75
Figure 4.20. Average values and 95 % confidence interval of respirometric data in Reactor 2 (Test 22) 76
Figure 4.21. Average values and 95 % confidence interval of respirometric data in Reactor 3 (Test 21) 76
Figure 4.22. T-O_2, N-O_2 and C-O_2 results in Respirometric Test 9 (R1-22.11.2012) 78
Figure 4.23. T-CO_2, N-CO_2 and C-CO_2 results in Respirometric Test 9 (R1-22.11.2012) 78
Figure 4.24. T-O_2, N-O_2 and C-O_2 results in Respirometric Test 13 (R1-17.01.2013) 79
Figure 4.25. T-CO_2, N-CO_2 and C-CO_2 results in Respirometric Test 13 (R1-17.01.2013) 80
Figure 4.26. T-O_2, N-O_2 and C-O_2 uptake rate results in Respirometric Test 18 (R1-27.02.2013) 81
Figure 4.27. T-O_2, N-O_2 and C-O_2 results in Respirometric Test 18 (R1-27.02.2013) 81
Figure 4.28. Results in Respirometric Test 24 (R1-09.04.2013) 82
Figure 4.29. T-O_2, C-O_2 and N-O_2 uptake rate results in Respirometric Test 10 (R2-26.11.2012) 83
Figure 4.30. T-O_2, C-O_2 and N-O_2 results in Respirometric Test 10 (R2-26.11.2012) 84
Figure 4.31. T-O_2, C-O_2 and N-O_2 results in Respirometric Test 17 (R2-14.02.2013) 84
Figure 4.32. Results in Respirometric Test 19 (R2-06.03.2013) 85
Figure 4.33. T-O_2, N-O_2 and C-O_2 results in Respirometric Test 19 (R2-06.03.2013) 86
Figure 4.34. Results in Respirometric Test 26 (R2-17.04.2013) 87

Figure 4.35. N-O_2 results in Respirometric Test 26 (R2-17.04.2013) 87
Figure 4.36. Results in Respirometric Test 8 (R3-19.11.2013) 88
Figure 4.37. Results in Respirometric Test 25 (R3-16.04.2013) 89
Figure 4.38. Results in Respirometric Test 1 (CR-18.06.2013)..... 90
Figure 4.39. Results in Respirometric Test 14 (R1-24.01.2013) 92
Figure 4.40. Results in Respirometric Test 15 (R1-31.01.2013) 93
Figure 4.41. Chemical structure of ATU (left) and complex formed with Ag^+ ions (right) 94
Figure 4.42. Color change in respirometric test chambers (5, 6, 9, 10) (a) at beginning of Test 4 (b) at the end of Test 4 94
Figure 4.43. Results in Respirometric Test 4 (R1-10.07.2012) 95
Figure 4.44. T-O_2 results in Respirometric Test 23 (R1-01.04.2013)..... 96
Figure 4.45. T-O_2 Results of Control and 5 mg/L Ag chambers in Respirometric Test 3 (R2-05.07.2012) 97
Figure 4.46. T-O_2 results in Respirometric Test 16 (R2-06.02.2013)..... 97
Figure 4.47. T-O_2 results in Respirometric Test 17 (R2-14.02.2013)..... 99
Figure 4.48. Control and 2 mg/L Ag results in Respirometric Test 19 (R2-06.03.2013) 100
Figure 4.49. Results in Respirometric Test 2 (R3-02.07.2012) 101
Figure B. 1. Raw data of Test 1 (CR - 18 June 2012) 10-20-40 mg/L Ag 141
Figure B. 2. Raw data of Test 2 (R3 - 2 July 2012) 0.5-1 mg/L Ag 142
Figure B. 3. Raw data of Test 3 (R1 & R2 - 5 July 2012) 5 mg/L Ag..... 143
Figure B. 4. Raw data of Test 4 (R1 - 10 July 2012) 5-10 mg/L Ag 144
Figure B. 5. Raw data of Test 5 (CR - 18 October 2012) 5-10 mg/L Ag 145
Figure B. 6. Raw data of Test 6 (CR - 22 October 2012) 5-10 mg/L Ag 146
Figure B. 7. Raw data of Test 7 (R1 - 8 November 2012) 1-5 mg/L Ag..... 147
Figure B. 8. Raw data of Test 8 (R3 - 19 November 2012)..... 148
Figure B. 9. Raw data of Test 9 (R1 - 22 November 2012)..... 149
Figure B. 10. Raw data of Test 10 (R2 - 6 November 2012)..... 150
Figure B. 11. Raw data of Test 11 (R1 - 6 December 2012) 2 mg/L Ag..... 151
Figure B. 12. Raw data of Test 12 (R1 - 12 January 2013) 10-20-40-50 mg/L Ag..... 152
Figure B. 13. Raw data of Test 13 (R1 - 17 January 2013) 5 mg/L Ag..... 153
Figure B. 14. Raw data of Test 14 (R1 - 24 January 2013) 1-2-4-5 mg/L Ag..... 154

Figure B. 15. Raw data of Test 15 (R1 - 31 January 2013) 1-2-4-5 mg/L Ag.....155
Figure B. 16. Raw data of Test 16 (R2 - 6 February 2013) 0.5-1-1.5-3 mg/L Ag.....156
Figure B. 17. Raw data of Test 17 (R2 - 14 February 2013) 1-1.5-2 mg/L Ag.....157
Figure B. 18. Raw data of Test 18 (R1 - 27 February 2013) 2 mg/L Ag.....158
Figure B. 19. Raw data of Test 19 (R2 - 6 March 2013) 2 mg/L Ag.....159
Figure B. 20. Raw data of Test 20 (R1 - 13 March 2013).....160
Figure B. 21. Raw data of Test 21 (R3 - 14 March 2013).....161
Figure B. 22. Raw data of Test 22 (R2 - 21 March 2013).....162
Figure B. 23. Raw data of Test 23 (R1 - 1 April 2013) 2-3-4 mg/L Ag.....163
Figure B. 24. Raw data of Test 24 (R1 - 9 April 2013).....164
Figure B. 25. Raw data of Test 25 (R3 - 16 April 2013).....165
Figure B. 26. Raw data of Test 26 (R2 - 17 April 2013).....166

LIST OF TABLES

Table 2.1. Threshold inhibition concentration of inorganic pollutants for activated sludge and nitrification process 24

Table 2.2. Threshold inhibition concentration of organic pollutants for activated sludge and nitrification process 25

Table 3.1. Composition of stock Feed 1 42

Table 3.2. Composition of stock Feed 2 43

Table 3.3. Composition of stock Feed 3 43

Table 3.4. Notation in respirometric graphs 55

Table 4.1. Properties of respirometric tests in chronological order 70

Table 4.2. Properties of respirometric tests in chronological order (continued) 71

Table 4.3. Analytical results of Respirometric test 24 82

Table 4.4. Analytical results of Respirometric Test 26 86

Table 4.5. Comparison of Respirometric Test 1 and 12 91

Table 4.6. Analytical and respirometric results in Test 14 92

Table 4.7. Analytical and respirometric results of Respirometric Test 15 93

Table A. 1. Operational results of Reactor 1 –July 2012 114

Table A. 2. Operational results of Reactor 1 – August 2012 115

Table A. 3. Operational results of Reactor 1 – September 2012 116

Table A. 4. Operational results of Reactor 1 –January 2013 117

Table A. 5. Operational results of Reactor 1 – February 2013 117

Table A. 6. Operational results of Reactor 1 – March 2013 118

Table A. 7. Operational results of Reactor 1 – April 2013 118

Table A. 8. Operational results of Reactor 1 – May 2013 119

Table A. 9. Operational results of Reactor 1 – June 2013 119

Table A. 10. Operational results of Reactor 2 –July 2012 120

Table A. 11. Operational results of Reactor 2 –August 2012 121

Table A. 12. Operational results of Reactor 2 –September 2012 122

Table A. 13. Operational results of Reactor 2 –January 2013 123

Table A. 14. Operational results of Reactor 2 –February 2013 123

Table A. 15. Operational results of Reactor 2 –March 2013 124
Table A. 16. Operational results of Reactor 2 –April 2013 124
Table A. 17. Operational results of Reactor 2 –May 2013 125
Table A. 18. Operational results of Reactor 2 –June 2013 125
Table A. 19. Operational results of Reactor 3 –July 2012 126
Table A. 20. Operational results of Reactor 3 –August 2012 127
Table A. 21. Operational results of Reactor 3 –September 2012 128
Table A. 22. Operational results of Reactor 3 –January 2013 129
Table A. 23. Operational results of Reactor 3 –February 2013 130
Table A. 24. Operational results of Reactor 3 –March 2013 131
Table A. 25. Operational results of Reactor 3 –April 2013 132
Table A. 26. Operational results of Reactor 3 –May 2013 133
Table A. 27. Operational results of Reactor 3 –June 2013 133
Table A. 28. Operational results of Control Reactor –July 2012 134
Table A. 29. Operational results of Control Reactor –August 2012 135
Table A. 30. Operational results of Control Reactor –September 2012 136
Table A. 31. Operational results of Control Reactor –January 2013 137
Table A. 32. Operational results of Control Reactor –February 2013 138
Table A. 33. Operational results of Control Reactor –March 2013 138
Table A. 34. Operational results of Control Reactor –April 2013 139
Table A. 35. Operational results of Control Reactor –May 2013 139
Table A. 36. Operational results of Control Reactor –June 2013 140

LIST OF SYMBOLS / ABBREVIATIONS

Symbol	Explanation	Units used
μ	Specific Growth Rate	(d^{-1})
θ	Hydraulic Retention Time	(d)
θ_x	Sludge Age	(d)
Ag^+	Free silver ion	(mg L^{-1})
AgNPs	Silver nanoparticles	
AOB	Ammonia Oxidizing Bacteria	
ATU	N-Allylthiourea	(mg L^{-1})
b	Decay coefficient of microorganisms	(d^{-1})
BAP	Biomass-Associated Products	
BNR	Biological Nutrient Removal	
BOD	Biochemical Oxygen Demand	(mg L^{-1})
C/N	Carbon to Nitrogen ratio	
C-CO_2	Carbonaceous CO_2 production	(mg)
CDNB	1-chloro-2,4-dinitrobenzene	
C-O_2	Carbonaceous oxygen uptake	(mg)
COD	Chemical Oxygen Demand	(mg L^{-1})
CON	Colloidal Organic Nitrogen	(mg L^{-1})
CR	Control Reactor	
CSTR	Continuously Stirred Tank Reactor	
DNP	2,4-dinitrophenol	
DO	Dissolved Oxygen	(mg L^{-1})
DON	Dissolved Organic Nitrogen	(mg L^{-1})
EPS	Extracellular Polymeric Substances	
ES	Enzyme-Substrate	
F/M	Food to Microorganism ratio	
FA	Free Ammonia	(mg L^{-1})
FNA	Free Nitrous Acid	(mg L^{-1})
IC_{50}	Inhibitor concentration that causes 50% inhibition	

K	Half saturation constant	(mg L^{-1})
LB-EPS	Loosely Bound-Extracellular Polymeric Substances	
MBR	Membrane Bioreactor	
MLSS	Mixed Liquor Suspended Solids	(mg L^{-1})
MLVSS	Mixed Liquor Volatile Suspended Solids	(mg L^{-1})
N-CO_2	Nitrogenous CO_2 production	(mg)
NH_4-N	Ammonium nitrogen	(mg L^{-1})
N-O_2	Nitrogenous oxygen uptake	(mg)
NO_2-N	Nitrite nitrogen	(mg L^{-1})
NO_3-N	Nitrate nitrogen	(mg L^{-1})
NOB	Nitrite Oxidizing Bacteria	
OUR	Oxygen Uptake Rate	(mg $L^{-1}h^{-1}$)
PFR	Plug Flow Reactor	
PON	Particulate Organic Nitrogen	(mg L^{-1})
POTW	Publicly Owned Treatment Works	
R1	Reactor 1	
R2	Reactor 2	
R3	Reactor 3	
rbCOD	Readily biodegradable Chemical Oxygen Demand	(mg L^{-1})
ROS	Reactive Oxygen Species	
sbCOD	Slowly biodegradable Chemical Oxygen Demand	(mg L^{-1})
SCFB	Semi-Continuously Fed Batch (Reactor)	
SMP	Soluble Microbial Products	
SOUR	Specific Oxygen Uptake Rate	(mg $L^{-1}h^{-1}mg^{-1}$)
SRT	Solids Retention Time	(d)
SS	Suspended Solids	(mg L^{-1})
SVI	Sludge Volume Index	
TAN	Total Ammonia Nitrogen	(mg L^{-1})
TB-EPS	Tightly Bound-Extracellular Polymeric Substances	
TCMP	2-chloro-6-(trichloromethyl) pyridine	(mg L^{-1})
T-CO_2	Total CO_2 production	(mg)
TKN	Total Kjeldahl Nitrogen	(mg L^{-1})

$T\text{-}O_2$	Total oxygen uptake	(mg)
TOC	Total Organic Carbon	(mg L^{-1})
UAP	Utilization-Associated Products	
VSS	Volatile Suspended Solids	(mg L^{-1})
X_a	Active biomass concentration	(mg L^{-1})
Y_a	Yield coefficient of nitrifying bacteria	(mg mg^{-1})
Y_h	Yield coefficient of heterotrophic bacteria	(mg mg^{-1})

1. INTRODUCTION

The increase in industrialization gives rise to environmental pollution which mostly arises from heavy metals and organic compounds. Because of their toxic effects and tendency of accumulation through the food chain, heavy metals constitute a major threat to environment. In activated sludge treatment systems, they mainly cause the inhibition of sludge due to their toxic effects.

Silver is one of the heavy metal species which has been widely used nowadays. The demand for silver metal has increased gradually and usage area has shifted rapidly due to technological developments. Especially, developments in nanotechnology increased the usage of silver in consumer products such as food container, soap, socks, air filter, cosmetics, washing machine, cleaner and wound care products (Johnson et al., 2005; Choi and Hu, 2009). Moreover, it is a well-known fact that silver has antimicrobial properties and low-toxicity on mammalian cells. Therefore, it is used in medical applications, disinfection applications in swimming pools, hospital hot water systems and potable water systems (Mijnendonckx et al., 2013). At the end of the life cycle, these silver containing materials are wasted and some portion of silver enters sewage systems and adversely affects wastewater treatment and environment (Choi et. al. 2008).

Heavy metals have the ability to form complexes with organic and inorganic compounds in aqueous phase. Choi et al. (2008) stated that the free silver ion (Ag^+) is the most toxic silver species. The inhibitory effect of Ag^+ comes from its sorption to negatively charged bacterial cell wall, deactivation of the cellular enzymes and disruption in membrane.

Activated sludge contains many types of microorganisms, immobilized in extracellular polymeric substances (EPS) or matrices which are formed by polymers of proteins, polysaccharides, humic acids, and lipids. The EPS formation is influenced by many factors such as operational parameters and wastewater constituents. Composition of EPS matrix significantly affects sludge properties (Ni and Yu, 2012). The carbon to nitrogen (C/N) ratio of a wastewater or substrate is one of the factors that changes the EPS

composition of activated sludge (Durmaz and Sanin, 2001). The C/N ratio also changes the biosorptive capacity of heavy metals on activated sludge (Yuncu et. al. 2006). On the other hand, alteration of the C/N ratio shifts microbial populations. When the C/N ratio is increased, the organic loading to the system increases; heterotrophic bacteria compete with nitrifying bacteria for the limited oxygen and space; nitrifying population decreases with time. On the contrary, if an activated sludge system receives a wastewater which has a low amount of organic matter, nitrifying bacteria are enriched in the system (Ling and Chen, 2005). Therefore, the change in the C/N ratio of activated sludge affects not only the sorption capacity of heavy metals but also bacterial population in sludge and sensitivity of activated sludge against heavy metals.

The overall objective of this research is to investigate the inhibitory effect of silver on the performance of lab-scale activated sludge reactors that are fed with synthetic wastewaters in which the C/N ratios differ. The relationship between Ag inhibition and feeding of activated sludge is studied.

The method employed in this study includes the cultivation of activated sludge by feeding with synthetic wastewaters that have different C/N ratios and to examine the influence of Ag on these sludge types. The study is carried out in aerated semi-continuously fed batch (SCFB) reactors. The reactors are analyzed for Chemical Oxygen Demand (COD) and nitrogen removal by measuring COD and various nitrogen species, respectively. Also, in order to control the activated sludge process, pH, suspended solids (SS) and volatile suspended solids (VSS) analysis were done. The inhibitory effect of Ag was measured by respirometry. When respirometry tests were conducted, samples were also examined by analytical methods. COD, NH_4-N, NO_2-N, NO_3-N, SS, VSS, pH analyses were conducted in reactors and respirometric tests throughout the study.

2. LITERATURE REVIEW

2.1. Activated Sludge Process

2.1.1. Definition and Features of the Activated Sludge Process

Activated sludge process is a widely used biological treatment system and is the keystone of wastewater treatment. It was developed at the beginning of 20th century in the USA and England. The fundamental of the process is to degrade the waste under aerobic conditions. Due to the production of active microorganisms, it was entitled as activated sludge process. The activated sludge includes extensive microbial variation such as bacteria, protozoa, fungi, rotifers and possibly algae. However, when the removal of specific nutrients or compounds is desired, biological treatment can be accomplished by specific microorganisms (Tchobanoglous et al., 2003). The primary goal of activated sludge process is the removal of organic matter in wastewater. In addition to this, nitrogen and phosphorous removal by activated sludge has gained popularity in last decades.

Activated sludge systems are designed for removing soluble and particulate organic matter from the influent wastewater in an aeration tank. The aeration tank is a suspended-growth bioreactor which contains flocs of microorganisms called activated sludge. The activated sludge is kept in suspension in the reactor by mixing and aeration. The slurry in the aeration tank passes through the settling tank and bacterial flocs are separated from the treated slurry by settling. A liquid-solid separation unit is used for separating microorganisms from the process stream and producing an effluent which has a low amount of suspended solids. Some portion of settled bacterial flocs is returned to the aeration tank and the other portion is wasted. The sludge recycle line is used for balance the concentration of active microorganisms in the bioreactor. By recycling of sludge, the microorganism concentration in the aeration tank is maintained at high values (Rittman and McCarty, 2001). The slurry in the aeration tank is termed as mixed liquor. The solids in suspension are called mixed liquor suspended solids (MLSS) and the organic fraction of these solids is named as mixed liquor volatile suspended solids (MLVSS). Figure 2.1 shows the schema of typical activated sludge process.

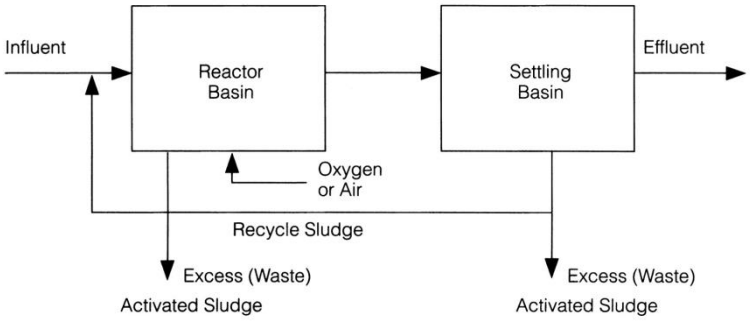


Figure 2.1.Scheme of typical activated sludge process

The solids retention time (SRT) is the most important design and control parameter of activated sludge. When Garrett and Sawyer (1951) used the idea of SRT in order to measure the performance of activated sludge, they measured the average retention time of cells in the reactor and linked it to the total suspended solids concentration. They observed that effluent water quality became poorer when SRT decreased (Rittmann and McCarty, 2001). For the activated sludge process an optimum SRT must be selected because microorganisms responsible for desired biochemical transformations should be retained in the reactor and grow. If sludge wasting rate is higher than the growth rate of microorganisms, microorganisms in the reactor will be washed out from the reactor and the system fails. The minimum SRT can be calculated for activated sludge systems and the design SRT should always be greater than this minimum.

Another important design parameter for activated sludge process is the food to microorganism (F/M) ratio or the process loading factor. F/M ratio can be defined as the mass of substrate applied per unit time per mass of microorganisms contained in the aeration tank (Grady et al., 2011). Equation 2.1 shows the F/M ratio and generally it is represented as g BOD or COD/g VSS.d (Tchobanoglous et al., 2003).

$$\mathrm{F/M\ Ratio} = \frac{\text{total applied substrate rate}}{\text{total microbial biomass}} = \frac{QS_0}{VX} \tag{2.1}$$

where Q is influent wastewater flowrate, m^3/d; S_0 is influent BOD or COD concentration, g/m^3; V is the tank volume, m^3; X is mixed liquor biomass concentration in the aeration tank, g/m^3.

The performance of activated sludge process depends on environmental conditions and operation of the system. In well-designed systems, flocculent biomass settles rapidly and compacts properly in the clarifier. Good bioflocculation is achieved by floc forming bacteria. SRT affects bioflocculation because it affects the growth of floc forming bacteria. SRT is the primary factor affecting the performance of activated sludge systems. (Grady et al., 2011).

Environmental conditions that affect the performance of activated sludge process are the dissolved oxygen level (DO), oxygen transfer, nutrients, pH and temperature. In activated sludge systems, DO level should not be below 2 mg/L O_2 in order to sustain aerobic conditions for microorganisms. The oxygen transfer systems are important for providing oxygen to the system and maintaining solids in suspension. Nutrients are necessary for growth of biomass. In the case of nutrient deficiency, filamentous organisms may become dominant in the system and floc forming bacteria cannot grow and can be washed out from the system. The temperature and pH affect the rates of biological reactions in the reactor. For this reason, optimum pH and temperature should be provided (Grady et al., 2011).

There are many types of reactor configurations in activated sludge process. These can be grouped into three. The simplest case of a continuous–flow suspended bioreactor is the continuously stirred tank reactor (CSTR). The mixing in the vessel is adequate to make the concentration uniform throughout the reactor and equal to the effluent concentration. The uniform conditions provide the same physiological state for microorganisms. The second type is the batch reactor. The batch reactor is a completely mixed reactor, but there is no flow through it. The substrate and biomass are placed into the vessel; then reactions proceed to completion. The third type is the plug flow reactor (PFR). The plug flow reactor may be considered as an infinite number of batch reactors. The practice of PFR is very difficult and usually approximations are made by considering the PFR as CSTRs in series (Grady et al., 2011).

2.1.2. Substrate Removal Mechanisms in Activated Sludge

Microorganisms need an energy source, a carbon source for synthesis of new cells and inorganic elements (nutrients) such as nitrogen, phosphorous, sulfur, potassium, calcium and magnesium. In Figure 2.2, bacterial populations are grouped with respect to cell carbon source and energy production. Organic matter and carbon dioxide are the carbon source for cell growth. Heterotrophic organisms use organic carbon for cell synthesis; on the other hand, autotrophs use carbon dioxide to form new cells (Tchobanoglous et. al, 2003).

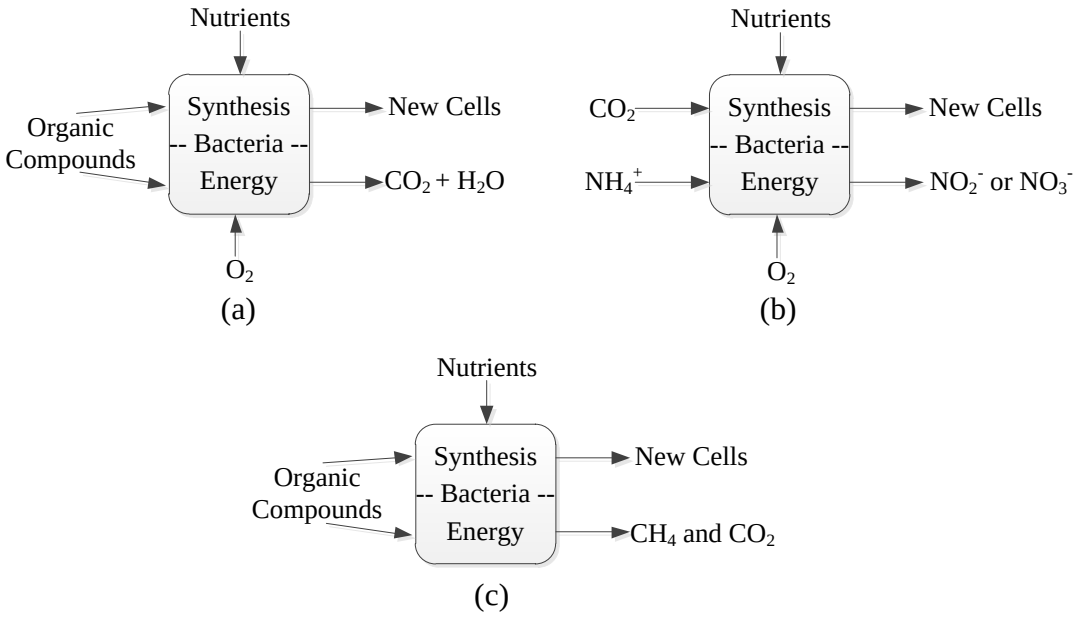


Figure 2.2. Bacterial metabolisms: (a) aerobic, heterotrophic, (b) aerobic, autotrophic, (c) anaerobic, heterotrophic (Tchobanoglous et al., 2003)

In the activated sludge process, pollutants serve as carbon and energy sources to microorganisms. Thus; they remove the pollutants from wastewater and produce new biomass, carbon dioxide, water and other forms (Grady et al., 2011).

2.1.2.1 Organic Carbon Removal. In the activated sludge process, organic matter is converted to carbon dioxide, water and other end products by heterotrophs. These reactions proceed in the presence of oxygen. The following example reflects the stoichiometry when

a casein-containing wastewater is used. According to Equation 2.2, while 184 g casein is consumed, 113 g new bacterial cells are produced. Since cell synthesis and energy reactions progress simultaneously, some portion of casein is used in cell synthesis and some portion of that is used for energy production (Rittmann and McCarty, 2001).

$$C_8H_{12}O_3N_2 + 3O_2 \rightarrow C_5H_7O_2N + NH_3 + 3CO_2 + H_2O \quad (2.2)$$

Wastewaters generally include many types of organic compounds and characterization of all compounds in the wastewater is difficult. There are three measures that are generally used for characterizing a wastewater: Biochemical Oxygen Demand (BOD), Total Organic Carbon (TOC) and Chemical Oxygen Demand (COD). COD is the most useful and reliable measure because it informs about the electron equivalent in the organic substrate, biomass and oxygen utilized. Also, it reflects the residual components of wastewater. Therefore, COD is the most preferred measurement (Orhon et al., 1997; Henze et al., 2000). For better design of activated sludge processes, wastewater composition must be assessed appropriately. Therefore, the COD fractionation of wastewater provides better process control and interpretation of the limits of activated sludge (Orhon and Çokgör, 1997; Fall et al., 2012).

Total COD in influent wastewater is divided into two groups: total nonbiodegradable (inert) COD and total biodegradable COD. Total inert COD is subdivided into soluble and particulate inert COD. The soluble inert COD in the influent wastewater can be easily determined because when influent wastewater enters the activated sludge system, soluble inert fraction is not affected from biochemical oxidation and passes through the system without any biochemical transformation. On the other hand, particulate inert COD follows different pathways. It is usually entrapped by activated sludge and discharged with the sludge wastage stream. The total biodegradable COD is divided into two subclasses: the readily biodegradable COD (rbCOD) and slowly biodegradable COD (sbCOD). The difference between the two fractions can be observed from biodegradation rates in experiments. When rbCOD is examined, two sub groups; fermentable and fermentation products can be seen. Besides this, sbCOD covers the particulate organics in the wastewater. Particulate organics cannot be taken up directly by microorganisms. They need to undergo hydrolysis, after that they can pass through the cell wall. Therefore, the

hydrolysis step becomes the rate limiting step for utilization of organics. The particulate organics cannot be considered in one group with respect to hydrolysis rate. They have a wide range of particle size distribution and complex structure. Therefore, sbCOD is grouped as rapidly hydrolysable and slowly hydrolysable COD. Total COD fractionation can be found in Figure 2.3 (Orhon and Çokgör, 1997).

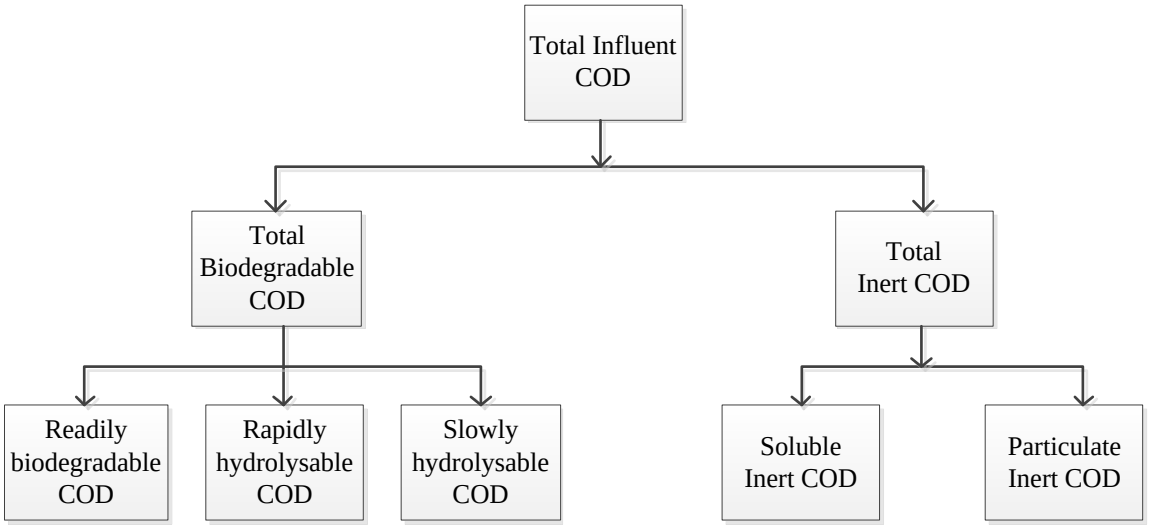


Figure 2.3. COD fractionation of wastewater (Orhon and Çokgör, 1997)

The substrate utilization of microorganisms can be examined on the basis of electron transport because respiration is an oxidation-reduction reaction. The substrate has the function of an electron donor and oxygen is the electron acceptor. When the substrate is consumed by microorganisms, some portion of electrons ($f°_e$) is transferred from substrate to oxygen (electron acceptor). These reactions provide energy to bacterial cells. The other portion of electrons ($f°_s$) is transferred to cells for production of new biomass. The sum of $f°_e$ and $f°_s$ is 1 and represents the total amount of substrate. In Figure 2.4, substrate utilization is shown. $f°_s$ can also be converted into mass units (g cell COD produced/g COD consumed). As shown in Equation 2.3 $f°_s$ can be converted to Y which is termed as the yield coefficient (Rittmann and McCarty, 2001):

$$Y = f°_s\,(\text{MW g cells/mol cells})/[(n_e\ e^-\ \text{eq/mol cells})/(8\ \text{g COD/}\ e^-\ \text{eq donor})] \qquad (2.3)$$

where MW is molecular weight of cells; n_e is number of electron an equivalents required to produce one mole of cells.

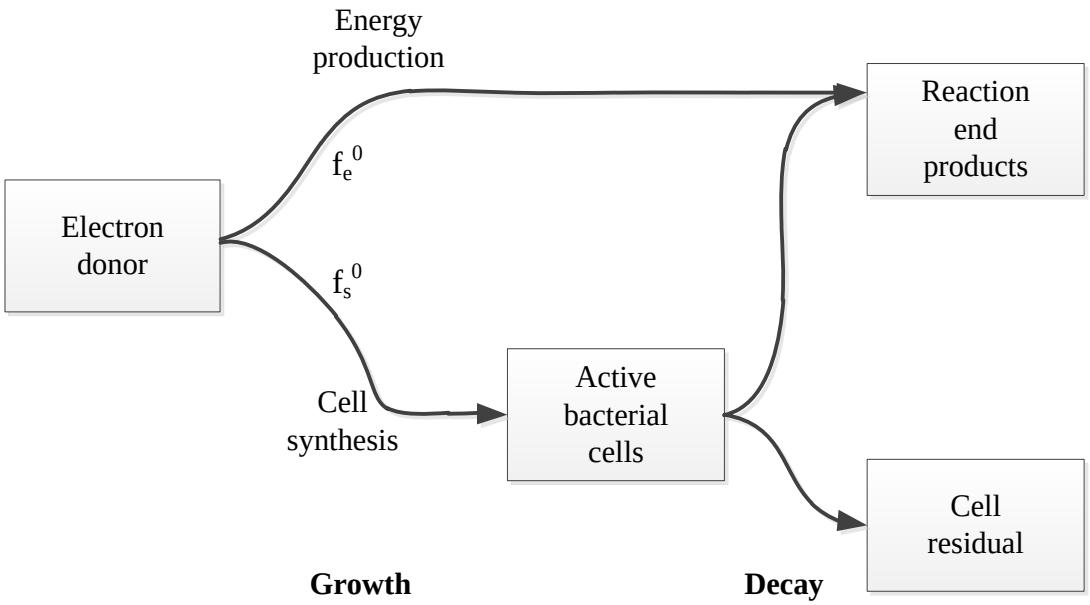


Figure 2.4. Substrate utilization for energy production and growth (Rittmann and McCarty, 2001)

As shown in Figure 2.4, the growth rate of microbial cells depends on substrate utilization and decay rate of biomass. The growth rate of biomass is usually expressed as Equation 2.4:

$$\frac{dX_a}{dt} = Y\left(\frac{-dS}{dt}\right) - bX_a \tag{2.4}$$

where dX_a/dt is the net growth rate (M/L^3T) of active microorganism (X_a, M/L^3); ($-dS/dt$) is the rate of substrate consumption (M/L^3T); b is the decay rate of microorganisms (1/T) and Y is the yield coefficient of microorganisms (M/M).

The specific growth rate of microorganisms can be expressed by the Monod equation in the following way:

$$\mu_{syn} = \left(\frac{1}{X_a}\frac{dX_a}{dt}\right)_{syn} = \hat{\mu}\frac{S}{K+S} \tag{2.5}$$

where μ_{syn} is the specific growth rate due to synthesis (T^{-1}), X_a is the concentration of active biomass (M/L^3), S is the concentration of the rate limiting substrate (M/L^3), $\hat{\mu}$ is the maximum specific growth rate (T^{-1}), K is the concentration giving one-half of the maximum rate (M/L^3) and t is time (T).

In the absence of substrate, the cells use themselves as an energy source in order to maintain their metabolic activities. This is represented as μ_{decay} and termed as endogenous respiration. b symbolizes the endogenous decay coefficient (T^{-1}).

$$\mu_{decay} = \left(\frac{1}{X_a}\frac{dX_a}{dt}\right)_{decay} = -b \tag{2.6}$$

Substrate utilization is mathematically represented in Equation 2.7

$$r_{ut} = -\frac{\hat{q}S}{K+S}X_a \tag{2.7}$$

where r_{ut} represents the rate of substrate utilization (M/L^3T) and $\hat{q}$ is the maximum specific rate of substrate utilization (M/M.T). Substrate utilization and biomass growth are related to each other:

$$\hat{\mu} = \hat{q}Y \tag{2.8}$$

In Equation 2.8, Y is true yield for biomass synthesis that is also expressed in Equation 2.3. In this way, net rate of biomass growth is demonstrated as:

$$r_{net} = -Y\frac{\hat{q}S}{K+S}X_a - bX_a \tag{2.9}$$

$$\mu = r_{net}/X_a = -Y\frac{\hat{q}S}{K+S} - b \tag{2.10}$$

Additionally, net rate of biomass growth is related to the SRT for a complete-mix activated sludge process.

$$\text{SRT} = \frac{1}{\mu} \tag{2.11}$$

Y is different for each type of microorganism. For heterotrophic microorganisms, Y value is represented as Y_h and is in the range of 0.42 – 0.49 g VSS/g COD. When the mass of microorganisms is measured in terms of COD unit (1.42 g cell COD/ g VSS), Y_h becomes 0.6-0.69 g cell COD/substrate COD. Also, $\hat{q}$ is in the range of 20-27 g COD/g VSS-d and $\hat{\mu}$ is between 8.4 and 13.2 d^{-1} for heterotrophic microorganisms (Rittmann and McCarty, 2001).

2.1.2.2. Nitrification. Nitrification is the oxidation of reduced nitrogen (NH_4^+-N) to nitrite-N (NO_2^--N) and nitrate-N (NO_3^--N). The nitrifying bacteria are autotrophs, chemolithotrops and obligate aerobes. These three properties give rise to significant differences between heterotrophs and nitrifiers. Primarily, autotrophs have the ability to fix and reduce inorganic carbon. This requires more energy than heterotrophic activity. The chemolithotrophic organisms have small $f°_s$ and Y values because nitrogenous compounds that are used as an electron donor, give less energy per electron equivalent than organic electron donors. Lastly, nitrifiers use O_2 as a direct reactant for initial monooxygenation of NH_4^+ to NH_2OH (hydroxylamine). Also, nitrifiers are severely affected by oxygen limitation (Rittmann and McCarty, 2001).

Nitrification is a two-step process and proceeds by ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). In the first step, NH_4^+ is converted to NO_2^- by AOB according to the following reaction (Tchobanoglous et al., 2003). This step is also called as nitritation.

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \tag{2.12}$$

The second step of nitrification is the conversion of NO_2^- to NO_3^-. The reaction is carried out by NOB. This step is named as nitratation.

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad (2.13)$$

The total nitrification reaction is expressed as follows:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \quad (2.14)$$

The equations that are found above are simple expressions of nitrification. When energy and biomass synthesis reactions are considered, the overall nitrification reaction can be written as follows:

$$NH_4^+ + 3.300\ O_2 + 6.708\ HCO_3^- \rightarrow 0.129\ C_5H_7O_2N + 3.373\ NO_3^- + 1.041\ H_2O + 6.463\ H_2CO_3 \quad (2.15)$$

According to Equation 2.15, 3.30 mg O_2 is required for the removal of 1 mg NH_4. When the calculations are made on the basis of NH_4-N, this value becomes 4.24 mg O_2/mg NH_4-N. When only substrate utilization is considered, oxygen consumption is approximated as 4.33 mg O_2/mg NH_4-N. 3.22 mg O_2 is consumed by AOB and 1.11 mg O_2 is consumed by NOB. For each mg NH_4-N removed, 0.146 mg biomass is produced due to AOB and 0.020 mg biomass produced due to NOB. In addition, alkalinity is an important parameter for nitrification. When influent wastewater has a low alkalinity and pH is not controlled, pH of activated sludge decreases sharply and this affects both heterotrophic and autotrophic activity. Due to stoichiometric balance, 7.14 mg alkalinity as $CaCO_3$ is required for the removal of 1 mg NH_4-N (Grady et al., 2011). However, in real cases alkalinity consumption may change in activated sludge systems due to solid retention time and predominance of other microbial species in the sludge (Benninger and Sherrard, 1978).

The unoxidized forms of nitrogen in a wastewater are measured by Total Kjeldahl Nitrogen (TKN). TKN includes the organic nitrogen and ammonium nitrogen. Approximately 60 to 70 percent of TKN in influent domestic wastewater is ammonium nitrogen (NH_4-N) and can be directly used for cell synthesis and nitrification (Tchobanoglous et al., 2003). Organic nitrogen may be divided into three parts: particulate organic nitrogen (PON), colloidal organic nitrogen (CON) and dissolved organic nitrogen (DON). This fractionation is based on filtration through two membrane filters; 0.1 and 1.2

µm pore size filters. DON is defined as the part of organic nitrogen below 0.1 µm. CON represents the organic nitrogen between 0.1 and 1.2 µm size. Finally, PON shows organic nitrogen compounds above 1.2 µm. Each major fraction has two sub-divisions like biodegradable and nonbiodegradable. According to ASM1 and ASM2d models, organic nitrogen has only two fractions; dissolved and particulate without a clear boundary between them (Henze et al., 2000). CON fractionation is important for full-scale systems. Since in lab-scale activated sludge systems usually synthetic feeds are used, it can be assumed that all of the organic nitrogen is in soluble form. In Figure 2.5, nitrogenous constituents and possible conversion pathway for nitrogen is demonstrated (Makinia et al., 2011).

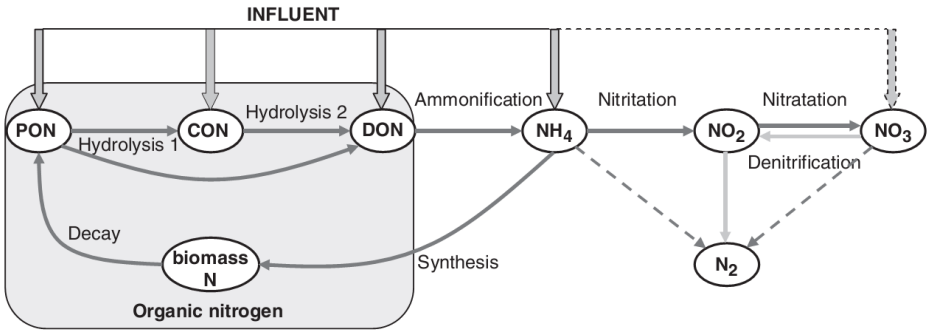


Figure 2.5.Nitrogenous compounds and N conversation pathways in activated sludge systems (Makinia et al., 2011)

Nitrification is affected by environmental conditions such as pH, temperature, DO level, alkalinity and inhibitors. When the temperature of the system is below 28°C, ammonia oxidation step is the rate limiting step. Therefore, the design of nitrifying systems should be made with respect to ammonia oxidation. The growth kinetics with respect to nitrogen is shown in Equation 2.16. It is here assumed that oxygen does not cause any limitation.

$$\mu_n = \hat{\mu}_n \frac{N}{K_n + N} - b_n \tag{2.16}$$

where μ_n is the specific growth rate of nitrifying bacteria (T^{-1}); $\hat{\mu}_n$ is the maximum specific growth rate of nitrifying bacteria (T^{-1}); N is the nitrogen concentration (M/L^3); K_n is the

concentration giving one-half of the maximum rate (M/L^3) and b_n is the endogenous decay coefficient for nitrifying organisms (T^{-1}) (Tchobanoglous et al., 2003).

The $f°_s$ and Y value of AOB are 0.14 and 0.34 g VSS/g NH_4-N, respectively. The $f°_s$ and Y value of NOB are 0.10 and 0.083, respectively. According to these values, $\hat{\mu}_n$ of the AOB and NOB is in the range of 0.32 - 1.02 d^{-1} and 0.34 – 1.1 d^{-1}. According to Equation 2.10, SRT of the system is the reciprocal of specific growth rate of microorganisms. When the specific growth rates of heterotrophs and nitrifiers are compared, nitrifiers have much lower specific growth rates. Thus a higher SRT is required for nitrification systems (Rittmann and McCarty, 2001). At low DO concentrations, nitrification is retarded and specific growth rate of nitrifiers is severely affected. For this reason, the expression of specific growth rate is modified as follows:

$$\mu_n = \hat{\mu}_n \left(\frac{N}{K_n + N}\right)\left(\frac{DO}{K_o + DO}\right) - b_n \qquad (2.17)$$

where DO is the dissolved oxygen concentration (M/L^3) and K_o is the concentration giving one-half of the maximum rate (M/L^3) (Tchobanoglous et al., 2003). Additionally, kinetic constants of nitrification change with respect to temperature and pH. Thereby, conversion for temperature and pH must be done.

2.1.3. Physical and Chemical Characterization of Activated Sludge

The liquid:solid separation plays a crucial role in activated sludge systems. Settling and compaction properties of activated sludge are as important as efficiency of the system because when the biomass cannot be separated from mixed liquor, the process of activated sludge is not completed. There are two main problems about settleability and compressibility, one of them is bulking sludge due to overgrowth of filamentous bacteria and second is poor flocculation of sludge (Jin et al., 2003). These problems arise from influent wastewater characteristics, process conditions, floc properties, microbial population and their activity (Henriques et al., 2005; Lee et al., 2003; Wilen and Balmer, 1999). Properties of activated sludge are directly related to floc characteristics. It can be examined in three aspects: morphological, physical and chemical properties.

The chemical properties of sludge are examined by polymeric constituents and metal content of sludge. High contents of Ca, Mg, Al and Fe in the sludge provide good settling and compressibility properties to activated sludge. Because bacterial flocs are highly negatively charged, they have a high affinity for cationic substances. When cations are attached on floc surfaces, they partially neutralize floc surfaces and influence the surface charge. Thereby, the compaction and settling properties of sludge are improved by the help of binding interactions between negatively charged flocs and cationic ions. Polymeric constituents of sludge are examined by extracellular polymeric substances (EPS) extraction and by the measurement of carbohydrate, protein and humic substances (Jin et al., 2003).

It is a well-known fact that microorganisms exists in biological treatment systems in the form of microbial aggregates such as sludge flocs, biofilm and sludge granules. The microbial aggregation is accomplished by polymeric networks known as EPS. The EPS covers the major component of organic fraction in activated sludge. When the EPS constitute 50–60% of the organic fraction, the cell biomass only make up 2–20% of the organic fraction in activated sludge (Sheng et al., 2010; Wilen and Lant, 2003). EPS are sticky solid materials secreted by cells and are involved in adhesion phenomena. Also, the formation of the matrix structure, the control of microbial physiology, and the long-term stability of sludge depends on the EPS composition of sludge. Therefore, it is considered that they have a significant role on the physicochemical properties of activated sludge (Ni and Yu, 2012). According to Jin and coworkers, a positive correlation was observed between sludge volume index (SVI) and the extracted EPS whereas no relationship was found between compressibility and EPS concentration. The high EPS concentration of the extracted EPS caused poor settleability and compressibility (Jin et al., 2003). Moreover, Sponza (2003) reported that the protein concentration of EPS influenced the settleability positively because sludge which has low SVI contains larger EPS based on protein. These studies show that EPS has a significant role on the physical and morphological characteristics of sludge because radical groups in EPS affect hydrophobicity of sludge and flocculating ability (Wilen and Lant, 2003). In order to understand effects of EPS on floc characteristics, microbial products in activated sludge must be analyzed.

The microbial products in activated sludge are classified in three groups; EPS, soluble microbial products (SMP) and inert biomass. In general term, EPS includes

macromolecules such as polysaccharides, proteins, nucleic acids, lipids and other polymeric compounds in activated sludge (Sheng et al., 2010). This complex formation mainly provides interaction and cooperation between cells in the microbial floc. Thus cells are held together and a stable floc formation occurs. The EPS in microbial floc is classified into two categories: bound and soluble EPS. Bound EPS contains sheaths, capsular polymers, condensed gel, loosely bound polymers, and attached organic material. Soluble EPS includes soluble macro-molecules, colloids, and slimes. Capsules which have condensed structure cover the cells. Slimes represents the loosely bond substances. Colloids represent the polymers that are not connected to cells (Laspidou and Rittmann, 2002). The structure of bound EPS is expressed by a two-layer model. The inner layer contains tightly bound EPS (TB-EPS) that binds to the cell surface tightly and has an appropriate shape. In the outer layer, loosely bound EPS (LB-EPS) exist. LB-EPS represents the loose slime layer without a certain boundary (Sheng et al., 2010).

SMP were defined as soluble organic compounds that are released from cells due to cellular activities like biomass growth and decay. They have significant role in biological treatment processes because they always exist in the system and form the major part of effluent COD and BOD. SMP are divided into two categories: substrate-utilization-associated products (UAP) and biomass-associated products (BAP). UAP are formed during substrate metabolism and BAP are formed from biomass during decay (Barker and Stuckey, 1999).

The inert biomass consists of solid materials other than EPS. It covers cell debris, entrapped suspended solids and inorganic precipitates. EPS also includes products of cellular lysis and decayed cells, which is also classified as inert biomass. Therefore, it can be said that some portion of inert biomass exists in bound EPS (Laspidou and Rittmann, 2002).

Relation between these microbial products is investigated by the unified theory which is introduced by Laspidou and Rittmann (2002). According to this theory, soluble EPS and SMP are the same; BAP is formed by hydrolysis of bound EPS; BAP and UAP are returned to substrate utilization metabolism because they are biodegradable; bound EPS is

formed from substrate utilization; and dead cell debris is produced during the endogenous decay and form inert biomass.

2.1.4. Factors Affecting the Properties of Activated Sludge

Activated sludge characteristics are influenced by system operation and influent wastewater composition. DO concentration in the aeration tank and SRT give rise to a significant change in sludge characteristics (Wilen and Balmer, 1999). In addition, the carbon to nitrogen (C/N) ratio of wastewater, substrate type and the presence of toxic compounds in a wastewater mainly affect the physicochemical properties of activated sludge.

SRT determines the retention of bacterial cells in activated sludge systems. Different SRT values also influence the physicochemical and biological characteristics of activated sludge. Lee and coworkers (2003) demonstrated that physicochemical and biological properties of microbial flocs and supernatant may cause membrane fouling in submerged membrane bioreactor (MBR) at different SRT values (20, 40, 60 days). Results of the study showed that the size of colloids in the supernatant increased with SRT because flocs were broken at high SRT. Also, cell residuals and macromolecules were released. When the EPS composition was examined in relation to SRT; at high SRT values, carbohydrate concentration of EPS was high and protein concentration was low at high SRT. The composition of EPS is more important than the total quantity of EPS. Also, the hydrophobic property of sludge increased when SRT of the system increased. Furthermore, Masse et al. (2006) found approximately the same results when they evaluated the effect of SRT on the characteristics of submerged MBR sludge and suspended growth activated sludge. According to this study, in suspended growth activated sludge, effluent quality of the system was worsened because of filaments, protein and carbohydrate release whereas submerged MBR system was operated well. At high SRT values, floc size decreased in both type of sludge systems. Also, bound EPS values decreased. Lastly, a study conducted by Liao and coworkers (2001) supported the findings outlined above. In their research, hydrophobicity of sludge changed with SRT. At higher SRT values, sludge became more hydrophobic.

In another study, it was proposed that toxic materials that inhibited the activated sludge, caused changes in floc sizes. According to this research, activated sludge which had a low floc size was more sensitive to cadmium and 2,4-dinitrophenol (DNP) than sludge that had a larger floc size (Henriques et al., 2005).

The EPS composition of activated sludge may differ in the presence of heavy metals. The soluble EPS (SMP) have a greater adsorptive ability for heavy metals than bound EPS. When the surface charge of EPS is negative, EPS can bind with the positively charged organic pollutants or metal ions through electrostatic interaction. Moreover, proteins have a high binding strength and capability than humic substances. The soluble EPS have a higher fraction of proteins than bound EPS, and thus they may have a greater binding capacity than bound EPS (Sheng et. al. 2010).

The last factor which affects activated sludge characteristics is the composition of wastewater.

2.1.4.1. Impact of the C/N Ratio on the Characteristics of Activated Sludge. The C/N ratio of influent wastewater plays an important role in organic carbon removal, nutrient removal and sludge characteristics. Also, the C/N ratio affects EPS production and composition in activated sludge flocs (Durmaz and Sanin, 2001).

Durmaz and Sanin (2001) studied the effect of the C/N ratio on the production and composition of extracellular polymers. Three different synthetic wastewaters at different C/N ratios (in terms of COD to NH_4) were used as feeds for lab-grown activated sludges. In order to represent conventional domestic wastewater, a C/N ratio of 17.5 was selected. The second reactor set had a C/N ratio of 5 to represent carbon limited conditions and the third reactor set had a C/N ratio of 40 to represent nitrogen limited conditions. When results were examined, first of all alteration in MLSS and MLVSS concentrations was observed, MLSS and MLVSS concentrations increased with an increase in the C/N ratio. In addition, the C/N ratio of 5 provided EPS composition was rich in proteins but low in carbohydrates, whereas the C/N ratio of 40 led to an EPS composition that is poor in proteins but rich in carbohydrates.

In their second study, Durmaz and Sanin (2003) investigated the effect of the C/N ratio on the physical and chemical characteristics of activated sludge. Since previous research showed that the C/N ratio of wastewater had changed the overall composition of EPS, they proposed that the C/N ratio possibly had influenced the physical properties of sludge. According to research, when the C/N ratio of the feed increased, viscosity increased and hydrophobicity of sludge decreased. Also, the surface charge of activated sludge became more negative with an increase in C/N ratio. Moreover, Sanin et al. (2006) studied the effect of operational conditions (C/N ratio in the feed, SRT, cation types) on EPS production, flocculation capacity and dewatering of the sludge. The results showed that an increase in the C/N ratio had improved the total EPS production. When EPS compositions were examined, it was observed that a low C/N ratio had improved the proteins in EPS and a high C/N ratio had stimulated the carbohydrates in EPS. In addition to these, when the protein to carbohydrate ratio of EPS increased, dewaterability of sludge improved. The possible reasons of this are better flocculation and increase in hydrophobicity due to the protein part in EPS.

In addition to these, the C/N ratio in a wastewater considerably affects population dynamics of microorganisms in a biological treatment process. The researches show that an increase in the C/N ratio in wastewater retards the accumulation of nitrifiers and nitrification process progresses slowly. Also, the studies showed that when the acetate concentration in the feed solution was increased, NOB could not be kept in the system. The reason of that, since heterotrophs competed for oxygen and space with NOB, heterotrophs remained in the system and NOB were washed out (Okabe et al., 1996). Furthermore, another study showed that the increase in the C/N ratio had caused depletion in nitrogen removal efficiency (Zhu and Chen, 2001; Fontenot et al., 2007).

2.1.4.2. Impact of the Carbon Source in a Wastewater on the Characteristics on Activated Sludge. The other significant parameter that changes the characteristics of activated sludge is the carbon source in a wastewater.

Li and Yang (2007) studied about the influence of EPS, including the impact of LB-EPS and TB-EPS on flocculation, sludge settlement and dewatering ability. In order to observe differences, they used different carbon sources at different SRT values. When

experimental results were examined, the sludge that was fed on acetate had larger floc sizes than the sludge fed on glucose. Under the same operational situations, the glucose-fed sludge produced more EPS than acetate-fed sludge. The reason of this was explained by the different metabolic pathways of glucose and acetate causing differences in EPS production. The glucose metabolism is more complex and thus, more extracellular enzymes may be required for the degradation of glucose. When the effect of different carbon source on liquid:solid separation was investigated, the acetate-fed sludge had better flocculation and separation ability, with lower effluent suspended solids and SVI values than the glucose-fed sludge. Also, it was realized that the existence of LB-EPS in the sludge adversely affected the performance of flocculation and settleability.

Ye and Li (2011) studied the impact of three different carbon sources; glucose, acetate and starch, on EPS and characteristics of sludge. Also, they investigated flocculation, settling and dewatering ability of the sludge with the alteration in EPS contents and compositions at different influent carbon source. They observed no difference in floc size with respect to influent carbon source. When the EPS production was examined, it was observed that the sludge fed with acetate had more LB-EPS than glucose and starch and the sludge fed with glucose produce more LB-EPS than starch. The reason was explained as follows. Acetate is a readily biodegradable substrate and easy degradation of acetate resulted in a high level of extracellular enzymes. Additionally, no difference was observed in the settleability of sludges and no correlation was found between EPS and physicochemical properties of activated sludge.

2.2. Inhibition of Activated Sludge

In many cases municipal wastewater treatment plants receive a great amount of industrial wastewater. Mostly, these industrial wastewaters contain toxic chemicals such as heavy metals, pesticides, antibiotics, aromatic hydrocarbons and chlorinated solvents. These substances generally influence the performance of a wastewater treatment plant by inhibiting active microorganisms in the system (Rittmann and McCarty, 2001; Evans et al., 1998).

2.2.1. Inhibition Mechanisms

Enzymes are organic catalysts that are formed by microorganisms and used in order to accelerate reactions. Enzymes play a crucial role in all metabolic reactions. They are the largest and the most specialized protein groups in cells. In the reactions, the enzyme fits to active site and works on the basis of lock-and-key principle. The enzyme catalyzed reactions are expressed by the aid of a general theory which was developed by L. Michaelis and M. L. Menten. According to this theory, enzyme E firstly reacts with substrate S and forms an enzyme-substrate complex ES. After that, the enzyme-substrate complex breaks down and free enzyme and products P are formed. Reactions are considered reversible and governed by various rate coefficients, k as shown in Equations 2.18 and 2.19 (Rittmann and McCarty, 2001).

$$E + S \underset{k_1}{\leftrightarrow} ES \tag{2.18}$$

$$ES \underset{k_2}{\leftrightarrow} E + P \tag{2.19}$$

Using the Equations 2.18 and 2.19, the Michaelis-Menten equation is derived in order to express enzyme kinetics as shown in Equation 2.20. This equation defines the relationship between substrate concentration and reaction rate on the basis of substrate saturation concentration and maximum possible reaction rate.

$$v = v_m \frac{S}{K_M + S} \tag{2.20}$$

where S shows the substrate concentration, v_m is the maximum reaction velocity. K_M is called as the Michaelis-Menten coefficient and equals the substrate concentration at which the reaction velocity is one-half of the maximum velocity. It corresponds to the affinity between the substrate and enzyme (Rittmann and McCarty, 2001).

An inhibitor is a substance that decreases the velocity of enzyme-catalyzed reactions (Mosier and Ladisch, 2009). Due to properties of inhibitors and their different effects on microorganisms, inhibition is considered as a complex issue. For instance, the inhibitor

may compete with substrate for the enzyme active site and affect substrate utilization adversely. On the other hand, it may influence the general cell functions such as respiration and then reduced biomass cause the slow utilization of particular substrate (Rittmann and McCarty, 2001). Inhibition phenomenon can be investigated in three classes; irreversible, reversible and substrate inhibition (self-inhibition). Irreversible inhibition is the permanent inactivation of enzymes. Inhibitory substance usually reacts with enzyme and forms a complex. The enzyme-inhibitor reaction cannot be reversed and inhibitory effect cannot be destroyed. This type of inhibition is also called as toxicity.

In reversible inhibition, the chemical agents (inhibitor) reduce the enzyme activity and adversely affect the biological process. The chemical agent does not destroy the enzyme and the reactions can be reversed when the inhibitor is removed from medium. There are three types of reversible inhibition: competitive, noncompetitive and uncompetitive inhibition (Rittmann and McCarty, 2001).

Competitive inhibition occurs, when the inhibitor competes with the substrate for the same active site in the enzyme. In this type of inhibition, inhibitor-enzyme complex is inactive but it is in equilibrium with the active form of enzyme. When the substrate concentration in the medium is increased, the inhibitor is detached from enzyme active site and the effectiveness of inhibition decreases. Competitive inhibition model is shown in Equation 2.21.

$$v = v_m \frac{S}{K_M(1 + \frac{I}{K_I}) + S} \tag{2.21}$$

where I is the competitive inhibitor concentration and K_I is the competitive inhibition coefficient. As shown in Equation 2.21, competitive inhibition affects the Michaelis-Menten coefficient (Mosier and Ladisch, 2009).

In noncompetitive inhibition, the inhibitor binds the enzyme or enzyme-substrate complex randomly, reversibly and independently. In this way, the inhibitor does not affect the binding of substrate because it binds a site which is other than the active site for substrate. However, the resulting complex of enzyme-substrate-inhibitor (ESI) is inactive

and it cannot be broken down and product formation cannot occur. The inhibition model for noncompetitive inhibition is represented in Equation 2.22. As shown in Equation 2.22, noncompetitive inhibition directly affects the maximum reaction velocity, v_m (Mosier and Ladisch, 2009).

$$v = \frac{v_m}{(1 + \frac{I}{K_I})} \times \frac{S}{(K_M + S)} \tag{2.22}$$

The third type of reversible inhibition is uncompetitive inhibition. The inhibitor binds only enzyme-substrate (ES) complex not the active site of enzyme. When the substrate concentration increases, the effect of inhibition also increases because more substrate cause more ES complex formation and the inhibitor can bind to this complex. The uncompetitive inhibition is demonstrated in Equation 2.23. Uncompetitive inhibition changes both K_M and v_m value of the reaction (Mosier and Ladisch, 2009).

$$v = v_m \times \frac{S}{K_M(1 + \frac{I}{K_I}) + S} \times \frac{1}{(1 + \frac{I}{K_I})} \tag{2.23}$$

The last type of inhibition is substrate inhibition that is generally named as self-inhibition. In this type of inhibition, enzyme-catalyzed reaction is slowed by high substrate concentration. The self-inhibition kinetics is represented in Equation 2.24 (Rittmann and McCarty, 2001).

$$v = \frac{v_m}{(1 + \frac{S}{K_{IS}})} \times \frac{S}{\frac{K_M}{(1 + \frac{S}{K_{IS}})} + S} \tag{2.24}$$

2.2.2. Inhibition of Organic Carbon Removal

Influent wastewaters arriving at wastewater treatment plant sometimes contain toxic compounds and this affects treatment system adversely. Especially, toxic shock loads of industrial chemicals can cause upset events in treatment systems. Because of the presence

of toxic compounds, effluent quality of wastewater deteriorates and this is observed as an increase in TSS and COD values. For instance; phenol, heavy metals and organic electrophilic chemicals are some of the sources which influence COD removal efficiency. Also, high ammonia loadings and aromatic compounds lead to inhibition of activated sludge systems (Henriques et al., 2007). There are many inhibitory compounds which have toxic effects. In Table 2.1 and Table 2.2, a list of pollutants is shown with their threshold inhibition concentrations for activated sludge process. The list is published by the US Environmental Protection Agency (EPA) (EPA Local Limits Development Guidance, 2004).

Table 2.1. Threshold inhibition concentration of inorganic pollutants for activated sludge and nitrification process (EPA, 2004)

Inorganic Pollutants	BIOLOGICAL PROCESS	
	Concentration Inhibiting Activated Sludge (mg/L)	Concentration Inhibiting Nitrification (mg/L)
Ammonia	480	-
Arsenic	0.1	1.5
Cadmium	1-10	5.2
Chloride	-	180
Chromium (VI)	1	1-10 (as $(CrO_4)^{2-}$)
Chromium (III)	10-50	-
Chromium (Total)	1-100	0.25-1.9
Copper	1	0.05-0.48
Cyanide	0.1-5	0.34-0.5
Lead	1-100	0.5
Mercury	0.1-1	-
Nickel	1-5	0.25-5
Zinc	0.3-10	0.08-0.5

Table 2.2. Threshold inhibition concentration of organic pollutants for activated sludge and nitrification process (EPA, 2004)

Organic Pollutants	BIOLOGICAL PROCESS	
	Concentration Inhibiting Activated Sludge (mg/L)	Concentration Inhibiting Nitrification (mg/L)
Anthracene	500	-
Benzene	100-500	-
2-Chlorophenol	5-200	-
Cloroform	-	10
1,2-Dichlorobenzene	5	-
1,3-Dichlorobenzene	5	-
1,4-Dichlorobenzene	5	-
2,4-Dichlorophenol	64	64
2,4-Dinitrophenol		150
2,4-Dimethylphenol	40-200	-
2,4-Dinitrotoluene	5	-
1,2-Diphenylhydrazine	5	-
Ethylbenzene	200	-
Hexachlorobenzene	5	-
Naphthalene	500	-
Nitrobenzene	30-500	-
Pentachlorophenol	0.95-150	-
Phenanthrene	500	-
Phenol	50-200	4-10
Toluene	200	-
2,4,6-Trichlorophenol	50-100	-
Surfactants	100-500	-

Microorganisms require metallic elements such as iron, chromium, copper, zinc and cobalt in micro and macro levels for proper growth. Although macro and micro amounts of metals are necessary for biomass growth, high concentrations of these metals cause inhibition in a biological system (Tchobanoglous et al., 2003). Heavy metals which are at high concentrations, form complex compounds within the cells and cause cell destruction (Principi et al., 2006). Also, there are many organic compounds which cause difficulties in treatment systems. A high amount of synthetic organic chemicals, also called xenobiotic

compounds, can cause inhibitory problems in the activated sludge (Tchobanoglous et al., 2003).

2.2.3. Inhibition of Nitrification

Nitrification is the most important step of Biological nutrient removal (BNR) in wastewater treatment plants. Bacteria that are responsible for nitrification are more sensitive to environmental conditions and inhibitory substances than heterotrophic bacteria (Blum and Speece, 1991). As shown in Table 2.1 and Table 2.2, while conventional activated sludge process is affected by pollutants at a rather high concentration, the nitrification process is affected at very low concentrations (Juliastuti et al., 2003). There are numerous compounds such as heavy metals and organic compounds causing inhibition in nitrification systems (Kocamemi and Çeçen, 2007a; Kocamemi and Çeçen, 2007b; Çeçen et al., 2010a; Çeçen et al., 2010b; Kelly II et al., 2004; Juliastuti et al., 2003). Furthermore, there are several factors that affect the degree of inhibition; these are pH of the system, inhibitor concentration, species present, the suspended solids concentration, sludge age of the system, solubility of the inhibitor, the concentrations of other cations and molecules (Juliastuti et al., 2003). Since nitrification inhibition is a wide issue, it can be examined in subclasses.

2.2.3.1. Substrate Inhibition in Nitrification. Efficiency of biological nitrogen removal depends on the ability of nitrifying organisms to oxidize ammonia to nitrate. In domestic wastewater treatment plants, inhibition of nitrification due to nitrogen concentration may not cause problems because nitrogen concentration is very low in domestic wastewater compared with other industrial wastewaters and leachate. However, high nitrogen concentration in wastewater may cause incomplete nitrification in industrial and combined wastewater treatment plants. Therefore, it can be said that the most significant compounds that cause substrate inhibition in nitrification, are ammonium (NH_4^+) and nitrite (NO_2^-) (Anthonisen et al., 1976). High strength wastewaters such as fertilizer wastewaters and landfill leachate have very high ammonium concentrations. For instance, fertilizer wastewaters have ammonium concentration more than 500 mg NH_4-N/L. The main problem in this type of wastewater is the inhibition of nitrogen removal at high substrate concentration (Aktaş and Çeçen, 2001; Çeçen, 1996; Çeçen and Orak, 1996).

Two substrates of nitrification, ammonia and nitrite are found in solution in ionized and unionized forms. Unionized form of ammonia is called as free ammonia (FA) (NH_3) and unionized form of nitrite is called as free nitrous acid (FNA) (HNO_2). The equilibrium between ionized and unionized forms of these compounds is controlled by pH. When pH increases, unionized form of ammonia increases and when pH decreases unionized form of nitrous acid increases. These equilibriums are shown in the following equations. Equation 2.25 represents the ammonia equilibrium and Equation 2.26 shows the nitrous acid equilibrium (Anthonisen et al., 1976).

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O \tag{2.25}$$

$$H^+ + NO_2^- \leftrightarrow HNO_2 \tag{2.26}$$

High ammonia concentration in the wastewater and high pH lead to formation of free ammonia. Depending on concentration, free ammonia causes complete inhibition of ammonia and nitrite oxidation. Lower concentrations of free ammonia are less effective on the activity of AOB whereas they can exert inhibitory effects on NOB. Thus, the result is nitrite accumulation in the system (Anthonisen et al., 1976).

When ammonia oxidation occurs, hydrogen ions are released and pH decreases. pH reduction gives rise to a shift in equilibrium and nitrite is converted into free nitrous acid. Also, when the pH decreases, ammonia equilibrium shifts and free ammonia concentration decreases. Free ammonia reduction in the system provides oxidation of ammonium to nitrite and FA inhibition on NOB disappears. However, sometimes in the absence of free ammonia and presence of sufficient aeration, oxidation of nitrite does not occur although the conditions favor the complete nitrification. The reason is the low pH level. Ammonia oxidation causes pH reduction, and at very low pH level, nitrite equilibrium shifts and free nitrous acid is formed. In this situation, the activity of NOB is stopped by FNA inhibition. Inhibitory FA concentration for AOB is in the range of 10 to 150 mg/L while for NOB it is in the range of 0.1 to 1 mg/L. Additionally, FNA concentration which inhibits the nitrifying organisms is in the range of 0.22 and 2.8 mg/L. Concentrations higher than 2.8 mg/L cause more severe inhibition. Total ammonia and nitrite concentration of wastewater

can be found by analytical methods. However, free ammonia and free nitrous acid concentrations can be found by calculation as shown in Equation 2.27 and 2.28 at known values of pH, temperature, ammonia and nitrite concentrations:

$$FA = \frac{(TAN) \times (10^{pH})}{(K_b/K_w) + 10^{pH}} \quad (2.27)$$

where FA is the concentration of free ammonia nitrogen, mg NH_3-N/L; TAN is the concentration of total ammonia nitrogen, NH_3-N + NH_4-N mg/L; K_b is ionization constant for ammonium; K_w is ionization constant for water and K_b/ K_w ratio is exp (6344/273 + °C)

$$FNA = \frac{NO_2 - N}{K_a \times 10^{pH}} \quad (2.28)$$

where FNA is concentration of free nitrous acid nitrogen, mg HNO_2-N/L; NO_2-N is concentration of nitrite nitrogen, mg NO_2-N/L; K_a, ionization constant for nitrite, is expressed as exp (-2300/273 + °C) (Anthonisen et al., 1976).

2.2.3.2. Inhibition of Nitrification by Inorganic and Organic Compounds. Nitrification is the most problematic process in BNR. It is influenced by many inorganic (i.e. heavy metals) and organic (i.e. aliphatics, phenols, aromatics and halogenated compounds) compounds (Kocamemi and Çeçen, 2007a; Kocamemi and Çeçen, 2007b). When nitrification is inhibited, denitrification does not proceed and nitrogen removal fails completely. Therefore, research on this issue is very broad and the effect of many substances on nitrification inhibition is examined.

Blum and Speece (1991) made a research about toxicity of 50 to 100 chemicals on three different bacterial groups, aerobic heterotrophs, AOB and methanogens, in order to form a toxicity database. Only organic chemicals were used in the study that were important environmental pollutants and existed in the priority pollutant list of EPA. Chlorinated aliphatics, aromatics such as chlorinated benzenes, other benzene derivatives and phenols were used. According to results, AOB were more sensitive than aerobic

heterotrophs by about one order of magnitude. Also, it was shown that chlorinated compounds were more toxic to bacteria than other organic pollutants.

In another study, lab-scale activated sludge reactors were continuously exposed to micropollutants and effects of continuous exposure were examined. The micropollutants (organic xenobiotic compounds such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, surfactants, pharmaceuticals and heavy metals) are found in natural waters and they have carcinogenic or endocrine disrupting effects. Also, they are found in many industrial discharges or household discharges that contain pharmaceuticals, personal care products and surfactants. In this study, 10 organic xenobiotics and 2 heavy metals (benzene, 1,3,5-trichlorobenzene, phenol, pentachlorophenol, naphthalene, pyrene, 4-nonylphenol, 4-dodecylbenzenesulfonic acid, 2,4-dichlorobiphenyl, decachlorobiphenyl, cadmium and lead) were selected from the priority pollutant list in EU regulations. The results showed that micropollutants did not affect total biosolids production, settling properties and COD removal. However, they significantly affected nitrogen removal. In the presence of micropollutants, removal of ammonia nitrogen decreased from 82% to 29-37% (Dionisi et al., 2007).

Kelly II and coworkers (2004) studied the effects of toxic shock loads on nitrification. They used single pulse inputs of six different industrially important toxins: 1-chloro-2,4-dinitrobenzene (CDNB), cadmium, 1-octanol, DNP, alkaline pH, cyanide (weak metal complex form). All shock loads inhibited nitrification process to a different extent. CNDB shock influenced nitrification significantly. The nitrifying sludge recovered in terms of nitrogen removal in 17 days after a 50 mg/L CNDB shock. Cyanide is a well-known nitrification inhibitor. When it was loaded to the reactor, effluent ammonia concentration increased, but inhibition recovered after 2 days of shock loading. The results showed that cadmium and 1-octanol inhibited ammonia oxidation whereas it did not significantly affect nitrite oxidation. After cadmium shock loading, recovery of nitrification was observed after 2, 6 and 11 days at 17, 27 and 52 mg Cd/L concentration, respectively. However, pH 11, cyanide and CNDB inhibited both ammonia oxidation and nitrite oxidation.

Semerci and Çeçen (2009) worked on the inhibitory effect of continuous Cd loading in a nitrification system. When Cd was added at 1 and 2.5 mg/L concentration to the

system, nitrification was inhibited by 30% and 47%, respectively. When the influent Cd concentration was increased to 3.5 mg/L and 8 mg/L, inhibition did not increase. Due to increase in metal dosage, acclimation of microorganism took place and heavy metal resistant species dominated in the nitrification system. When Cd in the influent was 10 mg/L inhibited the nitrification system severely whereas nitrite accumulation was not observed in this system. Also, the same behavior was observed in the study of Kelly and coworkers (2004). After continuous Cd feeding, a recovery period was started which lasted about 37 days. After the recovery period, redosing of Cd at 10 mg/L and 15 mg/L did not affect the performance of the system as before (Çeçen and Semerci, 2009). According to Mertoglu et al. (2008), increasing Cd loadings cause diversity in ammonia oxidizers. When Cd loadings were increased in a continuous flow nitrification system, metal–tolerant ammonia oxidizing bacteria became dominant in the system. Microbial species shifted from AOB to *Nitrosospira sp.* which could tolerate cadmium loadings.

Çeçen et al. (2010) studied the respiration inhibition in nitrification in the presence of Zn, Cu, Ni and Co. The effects of these metals were investigated using an activated sludge enriched in nitrifiers. These metals are considered essential for biomass growth at low concentrations, but they usually exhibit toxic effects when present at mg/L levels. In this research, in order to observe inhibition, a respirometric procedure was used which bases on the principle of simultaneous O_2 and CO_2 measurement. For each metal, IC_{50} values which concentration cause 50 % inhibition at 4, 8, 12, 24 hour were found. They were expressed as the concentration causing 50% inhibition based on both O_2 consumption and CO_2 production. Speciation of metals in the form of total, free and labile metal was also taken into consideration and the inhibitory behavior was interpreted by calculating the IC_{50} values based on this speciation. The results of the study showed that IC_{50} values based on total metal should not be compared directly, but rather attention should be paid to the species that each metal forms, because the inhibitory effect of metals changed with speciation. Çeçen et al. (2010b) investigated also the individual inhibitory effects of heavy metals (Cd, Pb, Hg, Ag, Cr) on nitrifying sludge. They used the same measurement techniques. Complete inhibition of nitrifying sludge did not occur for each heavy metal dosing. At the maximum dose, the inhibition was about 64% for Cr^{3+}, 96% for Ag, 80% for Hg, 70% for Cr^{6+}, almost 100% for Cd. When the inhibitory effect of heavy metals is examined, it is observed that the most inhibitory metal is Ag with respect to total IC_{50}

values. Also, the IC_{50} values showed that Hg is less inhibitory than Ag because Hg initially formed complexes with ammonia. However, Ag exists mostly in the form of free ion or labile complexes which dissociate into free ion. On molar basis, toxic effect of Ag metal was one to two orders of magnitude higher than other metals.

2.2.3.3. Inhibition of Nitrification by Specific Inhibitors. Nitrification progresses simultaneously with organic carbon removal. If oxygen is utilized by nitrification in a BOD test, this can make wrong interpretation of data and the determination of carbonaceous BOD difficult. Because of this problem, nitrification control methods were developed that do not affect organic carbon removal. The nitrification control methods can be examined in two subclasses: sample modification methods and chemical methods (Young, 1973).

Since sample modification methods (pasteurization, acidification and chlorination) did not give good results, scientists searched for new methods in which the aim was to inhibit nitrifying bacteria. Chemical methods were developed in this way. Firstly, methylene blue was used at 3 to 4 mg/L for this purpose. This chemical gave good results and inhibited nitrification. However, it interfered in carbonaceous oxygen demand and nitrite determinations. For this reason, it was not accepted in BOD tests. Secondly, thiourea and allylthiourea were used in order to inhibit nitrification. The disadvantage of thiourea is that it is biodegradable and has a lesser prolonged effect (Young, 1973). Moreover, the required amount of thiourea is approximately ten times higher than the other nitrification inhibitor, 2-chloro-6-(trichloromethyl) pyridine (TCMP) (Hays and Forbes, 1974). Additionally, in BOD tests thiourea makes a complex with iodine and interferes in the azide modification of Winkler DO determination. On the other hand, N-allylthiourea (ATU) is a well-known inhibitor of nitrification and does not cause serious problems in the BOD method. The inhibition mechanism of ATU is that it selectively inhibits ammonia oxidation. 0.5 mg/L ATU is adequate for inhibiting nitrification in a BOD test. Also, in order to inhibit nitrification in activated sludge, 10 mg/L ATU was used and complete nitrification was observed (Ginestet et al., 1998). According to Young, 0.5 to 2 mg/L ATU is suggested for BOD tests, in respirometric tests, 5 mg/L ATU should be used, if samples contain carbonaceous BOD less than 500 mg/L. In addition to that, it is suggested that 10 mg/L ATU has to be used at higher carbonaceous BOD concentrations. The disadvantages

of using ATU are that concentrations above 2 mg/L ATU interfere in COD measurements and can adversely affect the azide modification of iodometric method. Moreover, since it is biodegradable compound in the long period, it is not always effective in nitrification inhibition within 5 day BOD measurement (APHA et al., 2004).

Another nitrification inhibitor is 2-chloro-6-(trichloromethyl) pyridine (TCMP) that is also known as N-Serve and nitrapyrin. It was manufactured in the early 1960s in order to prevent nitrification in soil. According to researches, it was found that TCMP selectively inhibits ammonia oxidation. Therefore, TCMP was accepted as a good inhibitor for BOD tests. The effective inhibitory concentration of TCMP depends on the characteristics of a wastewater sample. 2 mg/L TCMP is adequate for BOD determination in both domestic and synthetic wastewater; higher concentrations may be required for industrial wastewaters. In respirometric BOD tests, 5 mg/L should be used for typical domestic wastewaters and natural stream waters. 10 mg/L TCMP should be used in respirometer tests if wastewaters have a carbonaceous BOD greater than about 500 mg/L. On the other hand, TCMP can dissolve in organic substances and slowly hydrolyze to 6-chloropicolinic acid. It is slightly soluble in water and nonbiodegradable (Young, 1973). Furthermore, a suspicious issue is that TCMP adversely affects carbonaceous BOD measurement. However, researches indicated that TCMP does not inhibit organic carbon removal. When ATU and TCMP are compared, it is observed that there is no difference between their effectiveness. For this reason, both of them can be used in carbonaceous BOD determinations. However, since ATU is a biodegradable compound, it is not preferred for ultimate BOD determinations. On the other hand, ATU is soluble and its stock solution can be easily prepared. But, TCMP is generally used in solid form or commercial forms (not pure TCMP) can be used in BOD tests (APHA et al., 2004; Young et al., 2005).

2.3. Inhibition of Activated Sludge by Silver

2.3.1. Properties and Sources of Silver Metal

Silver (Ag) is a transition metal with the atomic number of 47 and the atomic weight of 107.87 g/mol. Silver is found in the environment in four oxidation states: 0, 1+, 2+ and 3+. Ag^{0} and Ag^{1+} are the most widely seen forms whereas Ag^{2+} and Ag^{3+} are rarely found

in nature. Silver is generally found in the form of sulfide complex in association with iron, lead, gold or tellurides. In surface waters, monovalent ion of silver is widespread as sulfide, bicarbonate, sulfate salts or adsorbed onto organic or inorganic materials. Also, silver forms complex ions with chloride and sulfate. These complexes are not soluble or slightly soluble under environmental conditions (Purcell and Peters, 1998).

Silver is found in the earth's crust in low concentrations. When the concentration of silver reaches higher values in soil, mining activities can be done. Worldwide silver demand increases day by day because of its useful characteristics. Silver has highest electrical conductivity in metals and can conduct heat. Also it is a precious metal that is used in jewelry and decorative objects; it has antimicrobial properties against microorganisms. Therefore, silver is used in different areas for commercial, industrial, medical, and decorative purposes (Purcell and Peters, 1998).

Silver metal is largely used in the photographic and photo-processing industry. According to the U.S. EPA report, more than 99% of photographic processing facilities discharge their wastewaters to publicly owned treatment works (POTWs) in the form of silver thiosulfate such as $Ag(S_2O_3)_2^{3-}$ and $Ag(S_2O_3)_3^{5-}$. Thiosulfate complexes are very stable and do not dissociate. When silver thiosulfate compounds enter wastewater treatment plants, they are converted to silver sulfide that precipitates in sewage sludge (Pavlostathis and Maeng, 1998). Adams and Kramer (1999) showed that silver forms stronger complexes with sulfide compared to other metals (Hg > Ag > Cu > Pb > Zn) and it competes with other metals for available sulfide. Silver remains complex with sulfide as long as sulfide concentrations are higher than silver concentrations. Moreover, according to Bard et al. (1976), a 100 mg/L silver thiosulfate did not have any toxicity on activated sludge whereas 10 mg/L of freely dissociable silver nitrate ($AgNO_3$) caused 84 % inhibition and 10 mg/L silver chloride (AgCl) caused about 43% inhibition in activated sludge. Also, silver has the ability of partitioning (sorption) to other particles such as organic, inorganic particles or sludge surfaces. Shafer and coworkers (1998) indicated that silver has the highest particle-water partition coefficient (K_d) among other metals. For this reason, silver can be removed in a well-operated POTW with efficiency above 90%. Pavlostathis and Maeng (1998) indicated that when the photo-processing influent wastewater containing 1.85 mg/L Ag was treated, the in filtered effluent, Ag concentration

was lower than the detection limit of 0.01 mg/L. Also, researches showed that Ag ions mostly bind to biomass in activated sludge slurry and it does not found in the liquid phase (Çeçen et al., 2009)

Silver is also used in other industries. For instance, metallic silver is used in electronics, jewelry, silverware, solder, bearings, medical and dental applications (other than X-ray processing). Other silver compounds such as silver oxide are used in batteries and catalysts; silver chloride is used in alloys and solders; silver nitrate is used in mirrors and silver cyanide is used in electroplating. Figure 2.6 shows that silver releases into the environment are mostly in the form of solid wastes such as electronic wastes, mirrors, photographic wastes and batteries. Additionally, silver in a wastewater is wasted with sewage sludge from wastewater treatment plants (Purcell and Peters, 1998). Besides that, Johnson et al. (2005) indicates that with technological developments, silver usage increases in many fields. Silver usage in the photo-processing industry is decreasing because of shifts to digital photography while silver solder in electronics is increasing due to restrictions in the use of lead-based solders. Silver jewelry industry has a stable popularity. Figure 2.7 shows the global silver cycle. According to Johnson and coworkers (2005), 11500 tons of Ag are released to the environment by different routes. Silver in the soil or landfill is immobilized. The fate and effect of silver in the aquatic environment needs further investigation (Purcell and Peters, 1998).

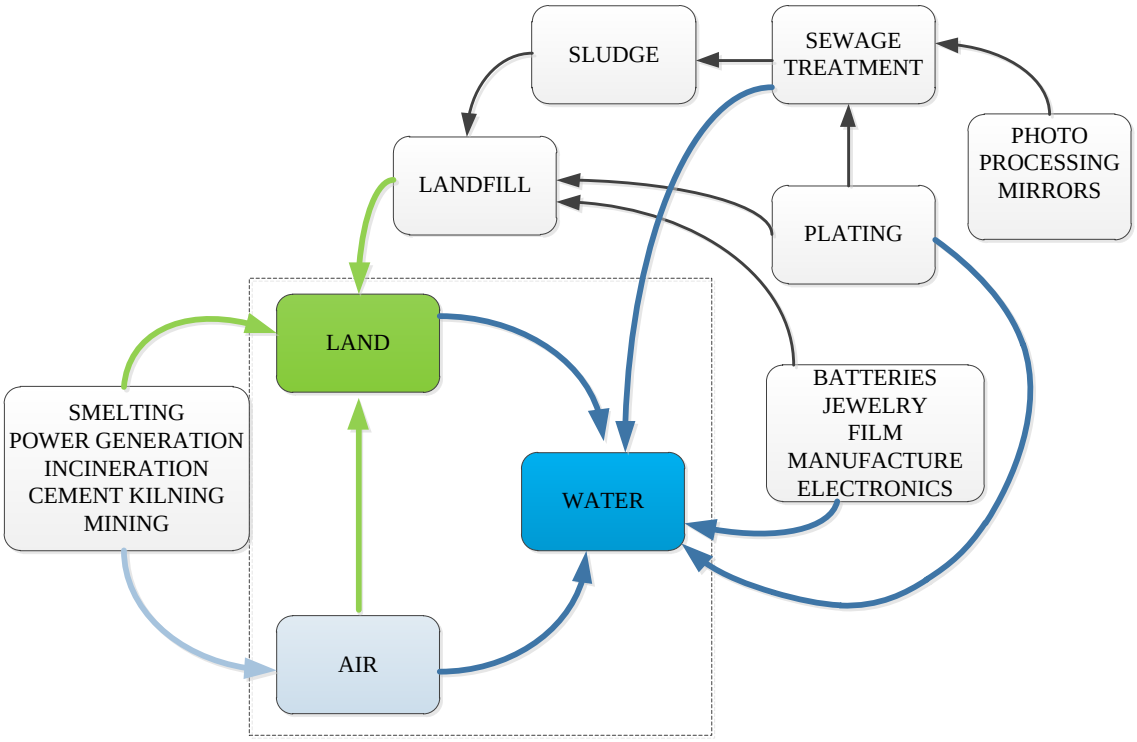


Figure 2.6. Schematic presentation of silver flow from industrial applications to the environment (Purcell and Peters, 1998).

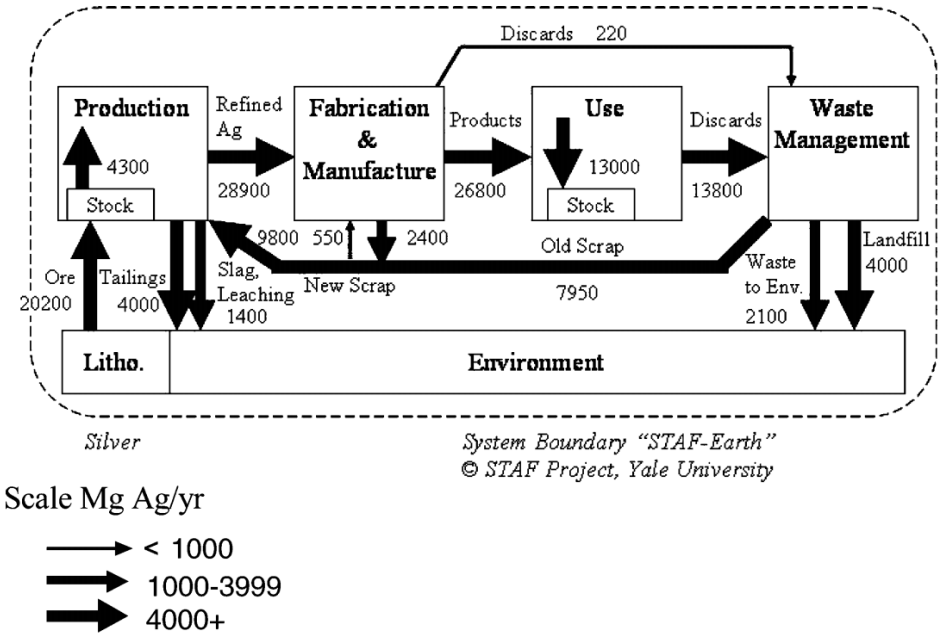


Figure 2.7. Global silver cycle, 1997 (All values are given in Mg Ag/yr "tonne Ag/yr"). (Johnson et al., 2005)

2.3.2. Inhibitory Effect of Silver

Antimicrobial properties of silver have been already known by Ancient Greeks. They used silver preparations for treatment of diseases, healing of wounds or preservation of foods and water. Additionally, it has been used as an antibiotic compound before the introduction of antibiotics in the 1940s. Nowadays, it is still used in medical applications for antibacterial properties. It is also used for disinfection purposes in swimming pools, hospital hot water system and potable water systems (Mijnendonckx et al., 2013).

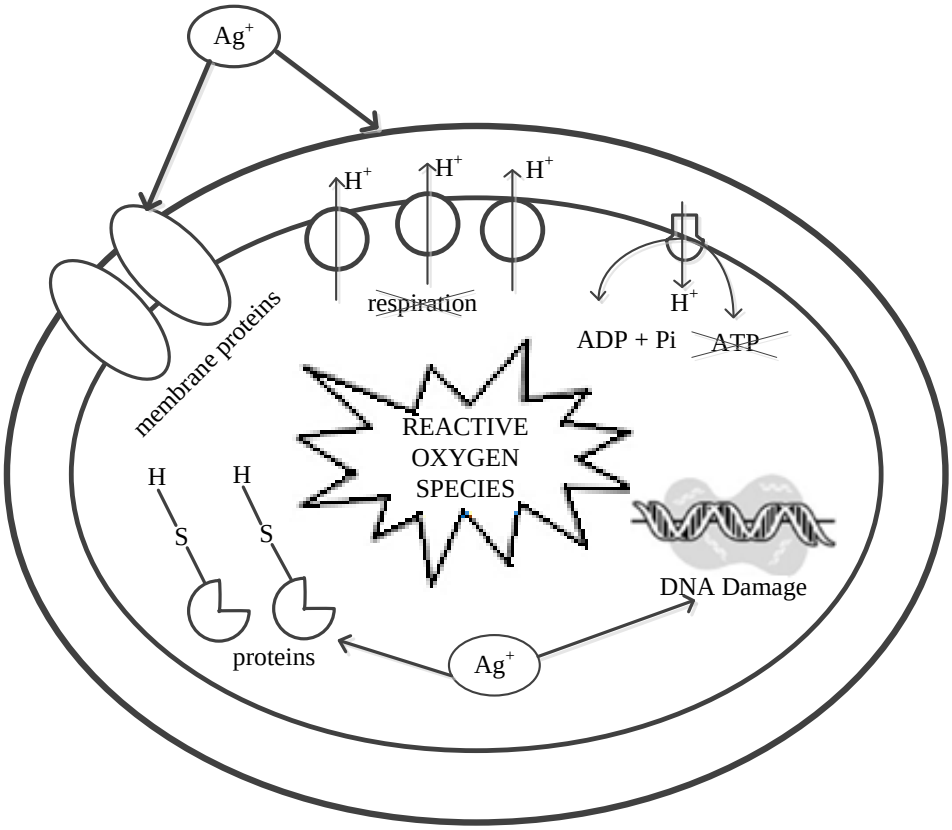


Figure 2.8. Antimicrobial effects of Ag^+ (Mijnendonckx et al., 2013)

As previously mentioned, some portion of silver is discharged to POTWs and like other heavy metals; silver may adversely affect biological treatment. Especially, it is known that free silver ion (Ag^+) is the most toxic silver species among other forms (Choi et al., 2008). The effect of Ag^+ ions on bacteria is explained in Figure 2.8. First of all, silver ions react with sulfhydryl groups present on the surface of microorganisms. Ag breaks the

H-S bonds and forms S-Ag bonds by replacing the hydrogen atoms. Therefore, respiration and electron transfer are completely blocked. The blockage of respiration gives rise to a collapse of the proton motive force that results in de-energizing of the membrane. Eventually, this may cause cell death. Silver ions rupture the membrane permeability and protons leak through the membrane. Destruction of cell membrane provides the entrance of Ag into the cytoplasm that can lead to additional damage. Under this condition, Ag^+ ions can complex with nucleic acids and interfere with DNA replication. Aerobic organisms produce reactive oxygen species (ROS) as a result of aerobic respiration. ROS are short-lived reactive oxidants that cause damage to proteins, DNA, RNA and lipids. Ag^+ ions stimulate ROS production of the cell and toxicity of silver is increased (Chen et al., 2012; Mijnendonckx et al., 2013).

There are numerous works about toxicity of silver compounds on microorganisms which are responsible for organic carbon and biological nutrient removal. Choi and coworkers (2008) studied the inhibitory effect of silver compounds on heterotrophic and autotrophic organisms. They used silver nanoparticles (AgNPs), Ag^+ ions ($AgNO_3$) and AgCl colloids. Enriched nitrifying bacteria were used as autotrophic organisms and *Escherichia coli PHL628-gfp* were used as heterotrophic bacteria. The results showed that 1 mg/L Ag as AgNPs, Ag^+ ions and AgCl colloids inhibited nitrifying bacteria approximately at 86 %, 42 % and 46 %, respectively. Also, nearly 0.5 mg/L Ag as AgNPs, Ag^+ ions and AgCl colloids inhibited E. coli approximately by 55 %, 100 %, 66 %. AgNPs affected nitrifying cultures more severely than heterotrophic cultures whereas Ag^+ ions blocked cell activity of bacteria. When the inhibitory effect of AgCl colloids was examined, it was observed that AgCl colloids might be inhibitory as other compounds. In another study, Choi and Hu (2009) studied the inhibition of nitrification by silver compounds. The results showed that AgNPs caused the most inhibitory effect on nitrifiers. Also, in this study the effect of anions on silver inhibition was investigated. Sulfate, chloride, EDTA, phosphate and sulfide anions were used in the experiment and results indicated that AgNPs and Ag^+ ions had similar complexation abilities with these anions. The presence of these anions in the wastewater may decrease the toxicity of silver compounds. However, silver compounds can form stronger complexes with biotic ligands on the cell membrane. For this reason, nitrification inhibition by silver compounds is noteworthy.

Liang and co-workers (2010) researched about the effects of toxic shock loads of AgNPs on activated sludge bacteria. Firstly, the results indicated that AgNPs have more toxic effects on nitrifying bacteria than Ag^+ ions. Also, AgNPs shock loads caused ammonia and nitrite accumulation in the system. When the effects of silver species on heterotrophic activity were investigated, it was shown that shock loading did not influence organic carbon removal.

Çeçen and coworkers (2010b) studied the inhibition of nitrification by silver ion. They also investigated the speciation of silver in wastewater and nitrifying sludge in detail. According to results, very low concentrations of silver of about 0.07 mg/L caused 10 % inhibition. Additionally, theoretical speciation of Ag showed that most of the silver was initially found in the form of free ion (Ag^+) in the nitrification medium. Labile complexes with ammonia were also present and could easily dissociate into free ion. In addition, a very small portion of silver was complexed with sulfates. The results indicated that silver had toxic effects on nitrifiers. Furthermore, when silver in wastewater was in contact with nitrifying sludge, most of the silver was bound to biomass.

Silver adsorption onto sludge affects sludge treatment and disposal such as sludge digestion and land use. Also, silver in the effluent is primarily found in free form and has a potential risk to receiving waters. In addition to these, Wang et al. (2003) conducted a study in order to determine the interaction of silver ions with wastewater constituents such as chloride, sludge particulates and dissolved organic matter. They proved that most of the silver reacts with chloride and adsorbs onto sludge. Complexation between Ag^+ ions and chloride depends on MLSS concentration, pH of the medium and concentration of dissolved organic matter. According to results, in acidic pH range, Ag^+ adsorption onto sludge increases with increasing in pH and decreases with high Ag^+ loadings. On the contrary, in alkaline pH range, Ag^+ adsorption decreases with increasing in pH and decreases with low Ag^+ loadings.

2.4. Respirometry of Activated Sludge and Respiration Inhibition

Respirometry is a useful technique in order to measure and interpret oxygen consumption of microorganisms under well-defined experimental conditions (Spanjers et al., 1998). Biological oxygen consumption (respiration) gives information about biomass growth and substrate removal. For this reason, respirometry is used as a tool for monitoring, modeling and control of the activated sludge process. Respirometers measure respiration rate of microorganisms, which is the mass of oxygen consumed per unit of time. There are various forms of respirometers from simple BOD test bottles to fully-automated instruments (Vanrolleghem, 2002).

Respirometers measure the DO in the liquid which is taken up by the biomass and thus oxygen uptake rate (OUR) of microorganisms can be interpreted. The DO measurements can be done by DO depletion in the liquid or gas phases. Direct DO measurements are done by electrochemical DO assessment methods and change of DO concentration in the liquid phase is assessed. Measurement of gaseous oxygen concentration is done by physical techniques such as paramagnetic, manometric and volumetric methods that measure the change of gaseous oxygen concentration (Vanrolleghem, 2002).

In the activated sludge process, the DO concentration change in the aeration tank gives rise to significant changes in process components such as the respiration of activated sludge (endogenous oxygen uptake rate) and the uptake of substrate or wastewater (exogenous uptake rate). Therefore, controlling DO concentration of activated sludge provides convenience in system operation. Also, respirometers can present information about endogenous and exogenous uptake rates of activated sludge (Ros, 1993).

Respirometers measure the decrease in DO concentration with time by using DO sensor. The relationship between the decrease in oxygen concentration and time is normally linear and the oxygen uptake rate is determined by calculations of the slope of the curve. OUR and generally reported as mg O_2/L.min or mg O_2/L.h. If the OUR value is related to the MLVSS concentration, the specific oxygen uptake rate (SOUR) is obtained. SOUR value represents the amount of oxygen used by known amount of microorganisms

and is reported as mg O_2/mg MLVSS.h. Additionally, when the measurement of endogenous oxygen uptake rate is desired, oxygen consumption of microorganisms is measured in the absence of substrate. Microorganisms maintain their metabolic activities at minimum level by degrading own cellular structure. For this reason, minimum OUR values are measured. On the other hand, if the test sample includes easily degradable substrates, after a test is started, maximum OUR is recorded. Under this condition, all bacteria are capable of degrading substrates and grow at maximum speed (Hagman and Jansen, 2007).

Nowadays, respirometry is widely used for both research and monitoring of wastewater treatment plants (Hagman and Jansen, 2007). Respirometry is primarily used for BOD measurement, determination of biokinetic constants in activated sludge, activated sludge modeling, assessment of the effect of a new wastewater on existing biological processes, determination of toxicity of wastewater on activated sludge (Mahendraker and Viraraghavan, 1995).

Respirometry can also be used for testing toxicity and detection of inhibitory compounds. Respirometry provides rapid detection of toxicity and also the detection of changes by doing continuous respiration tests (Hagman and Jansen, 2007). Researches show that respirometry is a rather simple and short tool for assessing toxicity. Also, respirometry can be used in assessing the toxicity of different municipal/industrial streams. (Dalzell et al., 2002; Ricco et al., 2004). According to Gutierrez and coworkers (2002), the respiration inhibition test is more reliable and rapid method for assessing toxicity. Also, respirometry is more representative test for toxicity evaluation when compared to Microtox®. The real activated sludge inhibition cannot be evaluated by Microtox® since Microtox® employs a single marine microbial species (*Vibrio fischeri*). On the other hand, in respirometric tests, a real mixed culture (activated sludge) sample is used as biomass.

3. MATERIALS AND METHODS

This study was designed to investigate the inhibitory effect of silver ion (Ag^+) on the performance of activated sludges. The main aim of the study was to obtain different sludge samples by changing the C/N ratio in the feed and to examine the effect of Ag on these sludges. The research was mainly performed in three phases:

- Set-up and operation of activated sludge reactors
- Determination of organic carbon removal and nitrification in different activated sludge reactors
- Respiration inhibition tests with Ag .

3.1. Materials

3.1.1. Synthetic Domestic Wastewater

In this study, three different synthetic wastewaters were prepared as “feeds” with respect to the COD/TKN ratio. This ratio is referred as the C/N ratio throughout the thesis. To all feeds, alkalinity was added for complete nitrification. Peptone water, glucose and acetate were used as organic substances in feeds. Peptone water contributed both to COD and TKN, while glucose and acetate contributed only to COD. The contribution of organic substances to COD and TKN was measured with analytical methods and checked with theoretical calculations.

Synthetic domestic wastewater 1 (Feed 1) was prepared as a stock solution having a COD/TKN ratio of 10 in order to feed Reactor 1. The COD/TKN ratio (or C/N ratio) in this feed was selected in accordance with typical domestic water. The composition of synthetic domestic wastewater 1 (Feed 1) is shown in Table 3.1.

The stock solution of Feed 1 had a COD of 10000 mg/L and TKN of 1000 mg/L. Approximately 20% of COD and 15 % of TKN came from peptone water. Ammonium sulfate in the feed contributed to most of the nitrogen (850 mg N/L) in the stock solution.

Reactor 1 was fed with this solution by diluting 20 folds before use throughout the experimental work. Then, the diluted solution had the strength of a typical domestic wastewater.

Table 3.1 Composition of stock Feed 1

Feed 1	**Name**	**Chemical Formula**	**Molecular weight (g/mol)**	**Concentration (mg/L)**
ORGANICS	D(+)-Anhydrous Glucose	$C_6H_{12}O_6$	180.2	5600
	Sodium acetate trihydrate	$CH_3COONa.3H_2O$	136.08	8000
	Peptone water			2000
INORGANICS	Ammonium sulfate	$(NH_4)_2SO_4$	132.14	4000
	Sodium bicarbonate	$NaHCO_3$	84.01	12000
	Di-potassium hydrogen phosphate	K_2HPO_4	174.18	1000
	Potassium dihydrogen phosphate	KH_2PO_4	136.08	1000
	Magnesium sulfate	$MgSO_4$	120.37	1000
	Manganese (II) sulfate monohydrate	$MnSO_4.H_2O$	169.02	25
	Calcium sulfate dihydrate	$CaSO_4$	172.17	500
	Iron sulfate heptahydrate	$FeSO_4.7H_2O$	278.01	343

Synthetic domestic wastewater 2 (Feed 2) was prepared as a stock solution having a COD/TKN ratio of 5 in order to feed Reactor 2. The composition of this wastewater is shown in Table 3.2.

The stock solution of Feed 2 had a COD of 5000 mg/L and TKN of 1000 mg/L. Approximately 7.5 % of TKN came from peptone water. Ammonium sulfate in the feed contributed to most of the nitrogen (975 mg N/L) in the stock solution. Reactor 2 was fed with this solution by diluting 20 folds before use throughout the experimental work. Then, the diluted solution had the strength of a domestic wastewater which has a lower organic content, but the same TKN strength as Feed 1.

Table 3.2. Composition of stock Feed 2

Feed 2	**Name**	**Chemical Formula**	**Molecular weight (g/mol)**	**Concentration (mg/L)**
ORGANICS	D(+)-Anhydrous Glucose	$C_6H_{12}O_6$	180.2	2800
	Sodium acetate trihydrate	$CH_3COONa.3H_2O$	136.08	4000
	Peptone water			1000
INORGANICS	Ammonium sulfate	$(NH_4)_2SO_4$	132.14	4360
	Sodium bicarbonate	$NaHCO_3$	84.01	12000
	Di-potassium hydrogen phosphate	K_2HPO_4	174.18	1000
	Potassium dihydrogen phosphate	KH_2PO_4	136.08	1000
	Magnesium sulfate	$MgSO_4$	120.37	1000
	Manganese (II) sulfate monohydrate	$MnSO_4.H_2O$	169.02	25
	Calcium sulfate dihydrate	$CaSO_4$	172.17	500
	Iron sulfate heptahydrate	$FeSO_4.7H_2O$	278.01	343

Table 3.3. Composition of stock Feed 3

Feed 3	**Name**	**Chemical Formula**	**Molecular weight (g/mol)**	**Concentration (mg/L)**
INORGANICS	Ammonium sulfate	$(NH_4)_2SO_4$	132.14	4714
	Sodium bicarbonate	$NaHCO_3$	84.01	12000
	Di-potassium hydrogen phosphate	K_2HPO_4	174.18	1000
	Potassium dihydrogen phosphate	KH_2PO_4	136.08	1000
	Magnesium sulfate	$MgSO_4$	120.37	1000
	Manganese (II) sulfate monohydrate	$MnSO_4.H_2O$	169.02	25
	Calcium Sulfate dihydrate	$CaSO_4$	172.17	500
	Iron sulfate heptahydrate	$FeSO_4.7H_2O$	278.01	343

Synthetic domestic wastewater 3 (Feed 3) was prepared as a stock solution having a COD/TKN ratio of 0 in order to feed Reactor 3. The composition of this wastewater is shown in Table 3.3. This feed consists only of inorganic compounds and does not contain any organic carbon. It is rich in terms of ammonium sulfate for enhancement of nitrification in Reactor 3. This stock solution contains 1000 mg NH_4-N/L.

3.1.2. Activated Sludge

In the first phase of reactor set-up, a single activated sludge reactor ("main reactor") was started up. Then, the sludge in this main reactor was divided into four reactors which were operated under different conditions.

In the first phase, 10 L of concentrated activated sludge was taken from the recycle line of the Paşaköy Advanced Biological Wastewater Treatment Plant on 25th of April 2012. The main activated sludge reactor having a volume of 19 L was started up. The reactor was aerated with air pumps and the compressed air line in the laboratory. The main activated sludge reactor was fed with Feed 1 at a loading rate of 500 mg COD/L.day and 50 mg TKN/L.day. The reactor was operated as a semi-continuously fed batch (SCFB) reactor. When the reactor reached steady-state conditions with respect to MLSS and MLVSS, the sludge was divided into four different reactors on 25th of May 2012. 3 reactors had a volume of 4 L (R1, R2 and R3) and one reactor had a volume of 9 L (CR). These four reactors were also fed as semi-continuously-fed batch reactors. Daily 1/20 of the sludge was wasted from the reactors to have a sludge age of 20 days. The configuration of these reactors is shown in Figure 3.1.

The Control Reactor (CR) was set up as a back-up of the main reactor. It was also fed with Feed 1 which had a C/N ratio of 10. The daily loading rate was as 500 mg COD/L.day and 50 mg TKN/L.day. Also, Reactor 1 (R1) was operated under the same conditions as the CR. Reactor 2 (R2) was operated with Feed 2 at a daily loading rate of 250 mg COD/L.day and 50 mg TKN/L.day. Reactor 3 (R3) was daily fed with Feed 3, nearly at a loading rate of 250 mg NH_4-N/L.day.

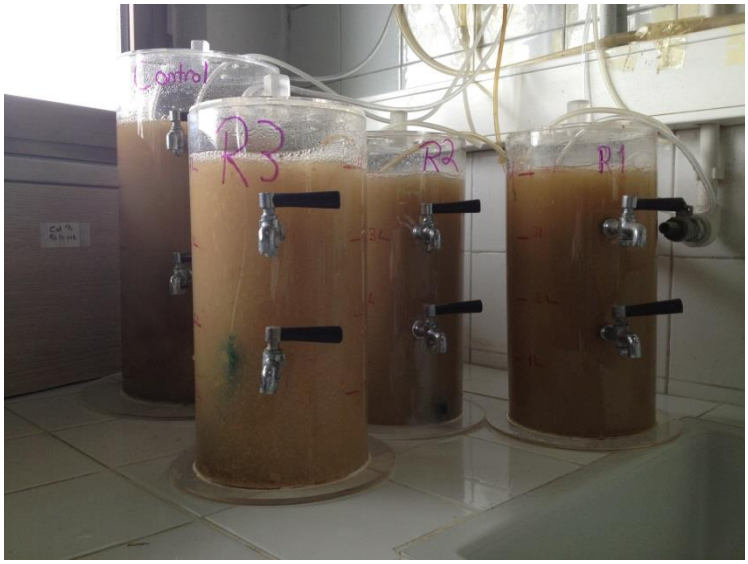


Figure 3.1. Configuration of reactors (R1, R2, R3 and CR)

3.1.3. Silver Used in Respirometry Tests

A commercial $AgNO_3$ solution was used throughout the study in respiration inhibition tests with Ag. For this purpose, Fluka Analytical 12818 Silver Standard for ICP was purchased. This standard solution had a concentration of 1000 mg/L $AgNO_3$ in 2% nitric acid. It was prepared with high purity Ag metal and HNO_3. Ag is found as Ag^+ ion (free silver ion) in this solution. When this solution was used in experiments, appropriate dilutions were made in order to work at the desired Ag concentration.

3.1.4. Nitrification Inhibitors

In respirometry tests nitrification inhibitors were used for distinguishing between carbonaceous oxygen demand ($C\text{-}O_2$) and nitrogenous oxygen demand ($N\text{-}O_2$) due to organic carbon removal and nitrification, respectively. Two different commercial inhibitors were used throughout respirometry tests.

N-allylthiourea (ATU) was the first nitrification inhibitor which was used in respirometry tests. The brand of the chemical is Fluka 06064 N-Allylthiourea. ATU stock solution was prepared in accordance with the OECD Test Guideline 209 and ISO 8192

International Standard. According to these standards, 2.32 g/L stock solution of ATU was prepared and the addition of 0.5 mL to a respirometry test sample resulted in a final concentration of 11.6 mg/L ATU (10^{-4} mol/L) in 100 mL test sample. This amount is adequate for complete inhibition of nitrification in a nitrifying activated sludge that has 1500 mg/L suspended solids. According to the Standard Test Methods for BOD Test, ATU stock solution should be preserved at 4°C and it is not stable for more than 2 weeks (APHA et al., 2004). For this reason, ATU stock solution was stored at 4°C and prepared biweekly throughout the experimental work.

Since complexation problems were observed between ATU and the Ag ion, it was decided to use another nitrification inhibitor. This specific inhibitor for nitrification used in respirometry tests was 2-chloro-6-(trichloromethyl) pyridine (TCMP). Two different commercial TCMPs were used throughout this study.

The first TCMP was Aldrich C1930 TCMP which a purity higher than 98 %. The first trial was the preparation of a stock solution with this TCMP. However, pure TCMP has a very low solubility in water. It is very difficult to prepare a known amount of stock solution (Young, 1973). Likewise, in this study we faced solubility problems in the first trial. In the second trial, TCMP was directly added to activated sludge samples. In the third trial, 0.05 g TCMP was dissolved in 10 mL of ethanol. It was then diluted to 1 L with deionized water. Thus, a 50 mg/L TCMP stock solution was obtained. This stock solution was added to test samples in order to reach a final concentration of 10 mg/L.

The second commercial TCMP was purchased from Hach. This product known as Nitrification Inhibitor Formula 2533 consists of 2% TCMP and 98% sodium sulfate. Since the purity of this product is low, the solubility in water is higher. Therefore, a stock solution could be prepared. According to the instructions on the product, 0.16 g TCMP should be added to 300 mL bottles in BOD tests. Regarding this information, 2.12 g Hach TCMP was added to 1 L deionized water and a 2120 mg/L stock solution was prepared. Since Hach TCMP was not pure, the real TCMP concentration in this stock solution was 42.4 mg/L. In respirometry tests, 25 mL of this stock solution were added to test samples in order to reach a final concentration of 10.6 mg/L.

3.2. Analytical Methods

The analytical methods used in the experiments are in accordance with *the Standard Methods for the Examination of Water and Wastewater* (APHA et al., 1998). The analytical methods are outlined below:

3.2.1. Chemical Oxygen Demand (COD) Analysis

In reactor operation and respirometry tests COD analyses were done in order to determine organic carbon removal. The chosen COD method is the dichromate closed reflux and colorimetric method. The principle of this method is the oxidation of organic matter by potassium dichromate under strongly acidic conditions. Samples were refluxed with a known excess amount of potassium dichromate ($K_2Cr_2O_7$) and H_2SO_4 for 2 hours at 150 °C in the ECO 25 Thermoreactor COD digester in the presence of Ag_2SO_4 as catalyst and $HgSO_4$ for preventing chloride interference. Oxidation of organic matter results in the change of chromium from the hexavalent (VI) state to the trivalent (III) state. Both of these species are colored and absorb in the visible region of the spectrum. Chromic ion absorbs strongly in the 600 nm region, where the dichromate has nearly zero absorption. Therefore, digested samples were measured colorimetrically at 600 nm with the Hach DR3900 Spectrophotometer. Calibration curves were prepared by using a KHP standard (Potassium hydrogen phthalate) solution. Additionally, in COD analyses three parallels were used and the average of triplicate results was reported.

Since activated sludge reactors contain biomass, COD analysis was done after filtration of test samples through 0.45 µm Whatman GF6 Glass fiber filters. In this way, contribution of biomass to COD results was prevented.

3.2.2. NH_4-N Analysis

NH_4-N analyses were done by Nessler Method which is accepted by US EPA for wastewater analysis and adapted from *Standard Methods for the Examination of Water and Wastewater*. For this purpose, the Method 8038 in the Hach Water Analysis Handbook (5^{th} ed.) was followed. Hach DR3900 Spectrophotometer was used for NH_4-N analysis.

Because the measurement range in this method is 0-2.5 mg NH4-N/L, appropriate dilutions were done at the beginning of experiments. 3 drops of Hach Mineral stabilizer, 3 drops of Hach Polyvinyl alcohol dispersing agent and 1 mL Merck Nessler reagent were used for each test sample and blank.

3.2.3. NO_2-N Analysis

NO_2 -N analysis was done by the Ferrous Sulfate Method (Method 8153) with Hach DR3900 spectrophotometer. Nitriver® 2 Nitrite Reagent Powder Pillows were used. This method can determine nitrite in a range of 0-250 mg/L as NO_2^-.

3.2.4. NO_3-N Analysis

NO_3-N analysis was done by the Cadmium Reduction Method (Method 8039) with Hach DR3900 spectrophotometer. Nitraver® 5 Nitrate Reagent Powder Pillows were used. This method can determine NO_3-N in a range of 0-30 mg/L as NO_3-N. Also, in order to compensate for nitrite interference, the test sample was pretreated with 30 g/L Hach Bromine Water and 30 g/L Hach Phenol solution.

3.2.5. TKN Analysis

TKN analysis was conducted by the Method 8075 with Hach DR3900 spectrophotometer. However, pretreatment should be done by acid digestion before spectrophotometric measurement. For acid digestion, the Hach Digesdahl Unit was used. 25 mL sample was digested with 4 mL H_2SO_4 and 15 mL H_2O_2 at 440 °C. For spectrophotometric measurements, one drop of TKN indicator was added, then 8 N KOH was added dropwise until a blue color appeared in 3 mL of digested sample. After that, test samples were filled to 20 mL with deionized water. 3 drops of Hach Mineral stabilizer and 3 drops of Hach Polyvinyl alcohol dispersing agent were added to 20 mL test samples, the samples were adjusted to a final volume of 25 mL. Then, 1 mL Merck Nessler reagent was added to test samples and readings were done with the Hach DR3900 spectrophotometer. The same procedure was applied to the blank sample.

3.2.6. Alkalinity Analysis

Alkalinity measurements were done by the titration method. 0.02 N standardized H_2SO_4 solution was used as a titrant. A mixed indicator solution was used in titration which indicated that pH 4.5 was reached. The used titrant volume was recorded and the total alkalinity in mg/L $CaCO_3$ was calculated as shown as follows:

$$\text{Alkalinity } \left(\frac{\text{mg } CaCO_3}{\text{L}}\right) = \frac{\text{A} \times \text{N} \times \text{D}}{\text{mL of sample}} \times 1000 \quad (3.1)$$

where A is mL of titrant used to neutralize alkalinity to specific pH endpoint; N is normality of titrant and D is equivalent weight of $CaCO_3$, 50 g.

3.2.7. pH Analysis

pH values were measured by the WTW Inolab-1 pH meter. The calibration of the pH probe was done by using standard buffer solutions having pH values of 4, 7 and 10 before measurements.

3.2.8. MLSS & MLVSS Analysis

MLSS analysis was done by drying the residue on filter paper (Sartorius Stedim Biotech Glassfiber Prefilter 0.45 µm) for one hour at 103°C in the FN 500 oven. MLVSS analysis was carried out by igniting the residue after MLSS analysis for 30 minutes at 550°C in the Protherm muffle furnace. Throughout the study, all MLSS and MLVSS analyses were done in duplicates and the average values were reported.

3.3. Experimental Work

3.3.1. Monitoring of Activated Sludge Reactors

Activated sludge reactors were monitored in order to control the removal efficiencies of COD and NH_4-N and to be informed about physical conditions of reactors. Therefore, COD, NH_4-N, NO_2-N, NO_3-N, MLSS, MLVSS, pH, and alkalinity measurements were regularly done in reactors (R1, R2, R3 and CR) that were operated simultaneously.

Semi-Continuously Fed Batch (SCFB) operation is a simulation of the continuous-flow operation in batch type reactors. The objective of using SCFB operation was to provide simultaneous operation of R1, R2, R3 and CR reactors under the same operation conditions and to monitor the differences between these four reactors in terms of COD removal and nitrification.

Reactors were fed on Mondays, Wednesdays and Fridays. Since problems were faced in the enrichment of nitrifying sludge in Reactor 3, the feeding period was changed to once-a-day. The sludge age in reactors was controlled by wasting some portion of mixed liquor during the feeding period. With the regulation of sludge wastage, MLVSS values in reactors were kept nearly at a constant level.

3.3.2. Respirometry Tests

Respirometry tests were carried out according to OECD Test Guideline 209 "Activated Sludge Respiration Inhibition Test (Carbon and Ammonium Oxidation)" and ISO 8192 International Standard "Water Quality Test for Inhibition of Oxygen Consumption by Activated Sludge for Carbonaceous and Ammonium Oxidation".

The Columbus Oxymax ER-10 respirometer and OLS200 Grant Shaker were used in respirometry tests. The respirometer has two sensors for oxygen and carbon dioxide measurements. The oxygen sensor measures oxygen consumption with electrochemical methods. Carbon dioxide sensor measures carbon dioxide production with a single beam, non-dispersive IR spectrophotometer. The respirometer performs measurements in a closed

gas sensing loop. Throughout the measurements, the gas existing in headspace of the test chamber is circulated through the sensor and back to the test chamber for a fixed period of time. The diagram of gas flow in the respirometer is shown in Figure 3.2. ER-10 Respirometer performs a series of gas measurements and records the net increase or decrease in the concentration of the monitored gas. The change in gas concentration is computed with the knowledge of headspace volume and gas sensing loop volume. After that, volume of gas consumed or produced by sample in the test chamber is calculated and all of the measured data is sent to host computer. Additionally, consumption and production data are normalized by ER-10 Respirometer to standard conditions for temperature and pressure: 0°C, 760 mm Hg. Results are presented in milligrams (mg) per minute. During the test, ER-10 Respirometer communicates with host computer in order to control data collection and presentation. Additionally, by the aid of a software program in the host computer the configuration of the system can be done in accordance to number of test samples and gas sensor calibration, measurement of test chamber head space volume and various adjustments. ER-10 Respirometer is capable to take measurements directly up to 10 different test samples and give the real-time graphical data representation.

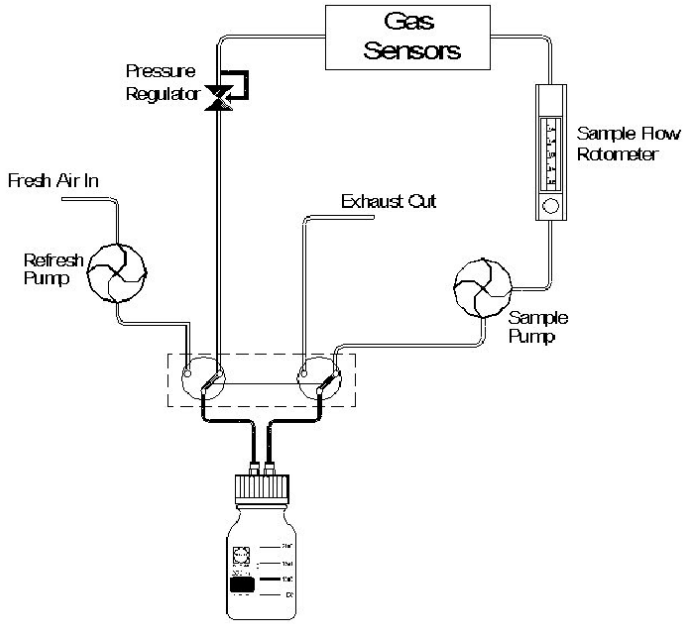


Figure 3.2. Block diagram of gas flow through ER-10 Respirometer

In respirometry tests, 10 different respirometric test chambers were operated as individual batch reactors. Two of them were generally chosen as control reactors in respiration inhibition tests. 20 mL sludge sample, necessary nutrients, minerals and various concentrations of $AgNO_3$ were added to test chambers. Additionally, if the measurement of carbonaceous oxygen consumption and carbon dioxide production was desired only, the nitrification inhibitor (ATU or TCMP) was added to test chambers. The final volume of all chambers was 100 mL. After the preparation of test samples, pH was adjusted to 7.5-7.6 in all chambers. Then, chambers were put in OLS200 Grant mechanical shaker at 25ºC, 120 rpm for 21 hour. The respirometric test configuration is shown in Figure 3.3.

All respirometric tests were also monitored analytically. For this purpose, pH, COD, NH_4-N were measured under initial and final conditions. Also MLSS and MLVSS analysis were done at the beginning of tests. Also, in some tests NO_2-N and NO_3-N analysis were done.

The following data were obtained with respect to time from respirometry tests:

- Oxygen (O_2) consumption rate (OUR): instantaneous oxygen uptake rate of activated sludge samples in the gas phase (mg/min)
- Cumulative oxygen consumption: the amount of oxygen consumed during respirometry tests (mg)
- Carbon dioxide (CO_2) production rate: instantaneous carbon dioxide production rate of activated sludge samples in the gas phase (mg/min)
- Cumulative CO_2 production: the amount of carbon dioxide produced during respirometry tests (mg)



Figure 3.3. Configuration of respirometry tests

3.3.2.1. Tests for Determination of the Sensitivity of Respirometric Data. The objective of these respirometry tests was to determine errors coming from preparation of test samples and the measurement sensitivity of ER-10 Respirometer. For that purpose, 10 identical test chambers were prepared. All chambers contained the same amount of sludge, feed solution and deionized water. In the ideal case, it was expected that data taken in all chambers were identical. However, differences were observed between chambers due to slight differences in sludge addition, feed addition and measurement errors in the respirometer.

3.3.2.2. Tests for Determination of Organic Carbon Removal and Nitrification. These respirometric tests were done in order to determine organic carbon removal and ammonium removal in Reactor 1 and Reactor 2. Since Reactor 3 was not fed with organic matter, it was enriched in terms of nitrifying bacteria. Organic carbon removal was not expected in this sludge.

To distinguish between carbonaceous oxygen demand and nitrogenous oxygen demand, ATU and TCMP were used in respirometry tests. ATU was used in two different ways. The first was the addition of ATU to test chambers at the beginning of respirometry tests (direct ATU). In the second method, activated sludge with a known MLSS and MLVSS was aerated with ATU for 15 hours before respirometry tests in order to inhibit nitrifying bacteria. After that the sludge was washed with fresh deionized water in order to

remove ATU from medium. This method is expressed as previous Pre-ATU. The idea was to prevent the complexation of ATU and Ag in respirometric tests.

TCMP was used in respirometry tests at various concentrations. Normally, 10.6 mg/L TCMP was used in respirometry tests. However, in order to determine the inhibition at higher concentrations, 21.6 and 31.8 mg/L TCMP were also used in tests. The concentration of TCMP stock solution was 42.4 mg/L. TCMP was added to the test chambers in solid form because test samples having a TCMP concentration of 31.8 mg/L were not prepared with this stock solution. Therefore, 0.16 g Hach TCMP was added to the test chambers in order to prepare a test sample having 31.8 mg/L TCMP concentration.

3.3.2.3. Respiration Inhibition Tests with Ag. These respirometric tests were done in order to determine effect of Ag on respiration of activated sludge. Various Ag concentrations were used in these tests.

3.3.2.4. Evaluation of Respirometry Data. Results of respirometry tests were represented graphically. Four graphs were obtained in respirometric measurements. These are oxygen uptake rate, cumulative oxygen uptake, carbon dioxide production rate and cumulative carbon dioxide production. Examples of raw data are shown in Appendix B. There are many notations on the graphs of respirometric data; these are expressed in the following Table 3.4.

According to raw respirometric data, the average values of parallel samples were calculated and T-O_2, C-O_2 and T-CO_2, C-CO_2 values were found as shown in the example below. Also, N-O_2 and N-CO_2 values were calculated by subtracting carbonaceous consumption or production from total consumption or production. The meaning of these abbreviations is as follows:

- T-O_2: Total oxygen uptake rate (mg/min)/cumulative oxygen uptake (mg)
- C-O_2: Carbonaceous oxygen uptake rate (mg/min)/ cumulative carbonaceous oxygen uptake (mg)
- N-O_2: Nitrogenous oxygen uptake rate (mg/min)/ cumulative nitrogenous oxygen uptake (mg)

- T-CO_2: Total CO_2 production rate (mg/min)/ cumulative CO_2 production rate (mg)
- C-CO_2: Carbonaceous CO_2 production rate (mg/min)/ cumulative carbonaceous CO_2 production (mg)
- N-CO_2: Nitrogenous CO_2 production rate (mg/min)/ cumulative nitrogenous CO_2 production (mg)

Table 3.4. Notation in respirometric graphs

NOTATION	EXPLANATION
Ch	Number of respirometric test chamber
R1, R2, R3 and CR	Activated sludge samples taken from the reactors
feed	Addition of feed solution (Feed 1, 2 or 3)
Control	Activated sludge samples containing only the feed solution
ATU	Addition of the nitrification inhibitor, ATU to test chamber
Pre-ATU	Aeration of activated sludge sample with the nitrification inhibitor, ATU before respirometric test
Direct ATU	Addition of ATU to respirometric chamber before the start of respiration test
TCMP	Addition of TCMP to test chamber (final concentration is 10.6 mg/L)
mg/L TCMP	Addition of TCMP to test chamber at various concentrations
(solid form) TCMP	Addition of TCMP to test chamber in solid form where final concentration is 31.8 mg/L
mg/L Ag	Concentration of Ag metal in the test chamber
R1, R2, R3, CR Sludge	Measurement of endogenous respiration (only activated sludge sample and deionized water)

Nitrogenous O_2/CO_2 values were calculated as follows:

$$N - O_2 = T - O_2 - C - O_2 \quad (3.2)$$

$$N - CO_2 = T - CO_2 - C - CO_2 \quad (3.3)$$

An example to this calculation is presented in Figure 3.4 and Figure 3.5. In these figures, total oxygen consumption (T-O_2) represents the oxygen consumption due to organic carbon removal and nitrification (C-O_2+N-O_2). In some respirometric chambers, C-O_2 was determined separately by the addition of a nitrification inhibitor. As shown in Figure 3.4, N-O_2 was then obtained by calculation.

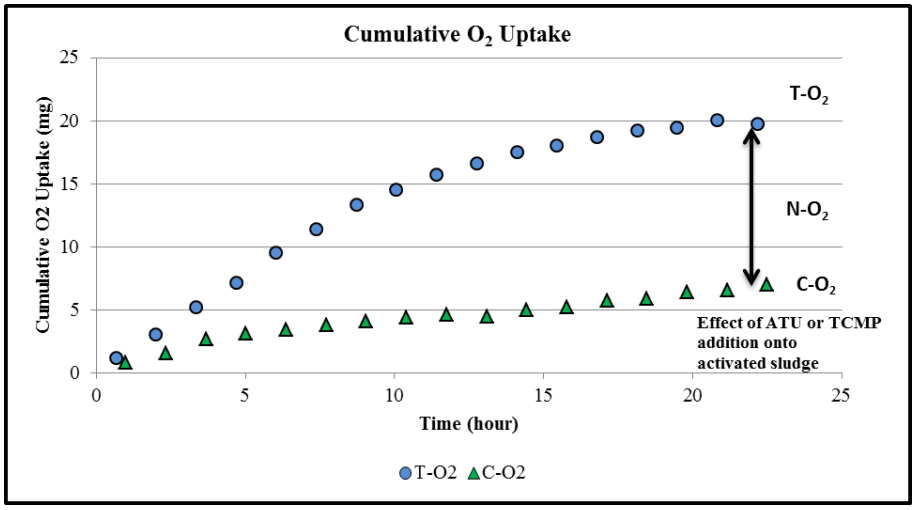


Figure 3.4. Example of N-O_2 calculation from raw respirometric data

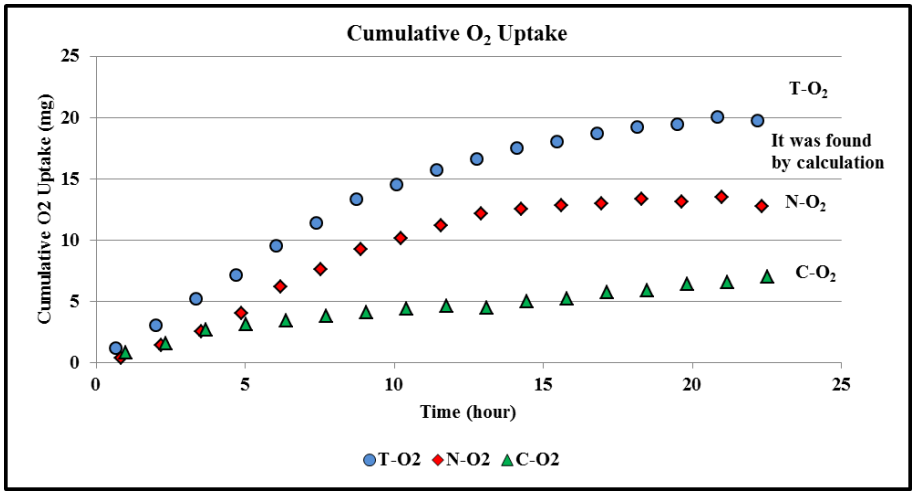


Figure 3.5. Example of graphical presentation of N-O_2

4. RESULTS AND DISCUSSION

4.1. Operation of Activated Sludge Reactors

In order to investigate the removal of organic carbon and nitrification in reactors at different C/N ratios, activated sludge reactors were started up as explained in "Materials and Methods" (Section 3.1.2). The main reactor had been in operation for one month. It was started-up on 25th April 2012 and operated until 25th May 2012. Figure 4.1 shows that the main reactor reached steady-state conditions in terms of MLVSS and MLSS. Operation of a reactor at steady-state condition indicates that the reactor has a constant physiological state. For this reason, it was decided that the main reactor was divided into four reactors. The results obtained in reactors are given in following sections.

In addition, these four activated sludge reactors were monitored in terms of EPS composition and production within the scope of a Ph.D. Thesis which investigates the relationship between metal inhibition and microbial products in biological systems (Geyik, 2013; Çeçen, 2013).

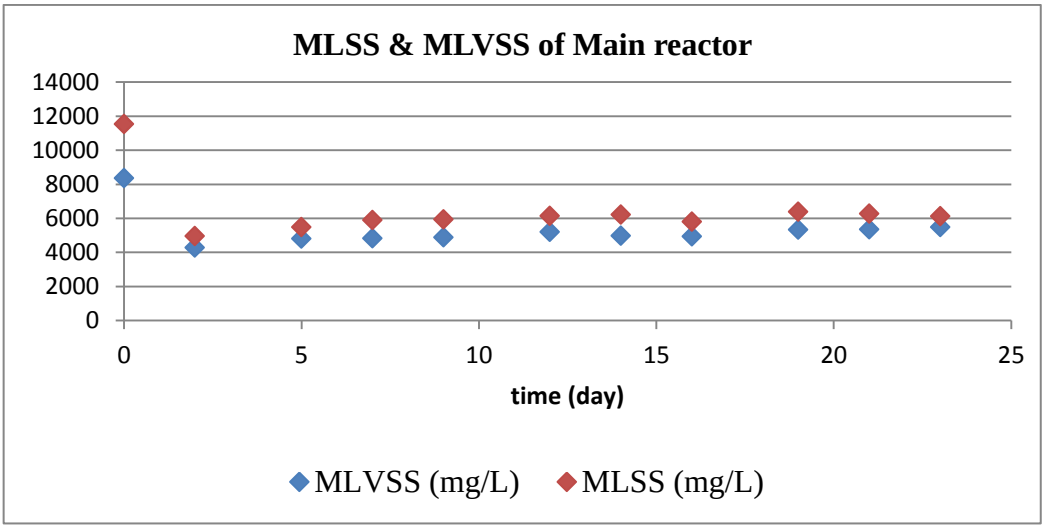


Figure 4.1. MLSS and MLVSS results in the main reactor

4.1.1. Removal of Organic Carbon in Reactors

Organic carbon removal in activated sludge reactors was measured with COD analysis. The detailed results about organic carbon removal in terms of influent COD, effluent COD and removal efficiency are presented in Appendix A. Influent values represents values which were measured at the initial condition of one SCFB run and effluent values show the concentration under final conditions. Monitoring of organic carbon removal is important since it demonstrates the performance of heterotrophic activity in an activated sludge system. The experimental period lasted from 25th May 2012 to about 29th June 2013 and SCFB runs were monitored continuously. However, in October, November and December 2012, data about activated sludge reactor are missing. For this reason, data were not edited in tabulated form.

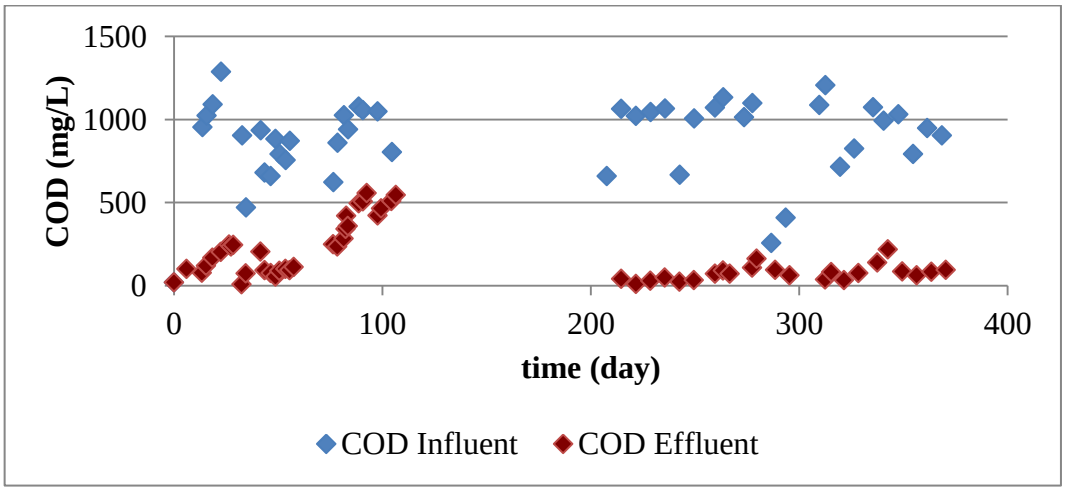


Figure 4.2. COD profiles in Reactor 1 (R1)

Control Reactor (CR) and Reactor 1 (R1) were fed with the same stock solution (Feed 1) and at the same organic loading as mentioned in Section 3.1.2. Figure 4.2 shows the influent and effluent COD in R1. When data belonging to January, 2013 were examined, removal efficiencies in R1 and CR were approximately 90 %. At certain periods, the activated sludge reactors were settled, their supernatants were wasted and reactors were washed with fresh water. After this washing procedure, high removal efficiencies were obtained in reactors.

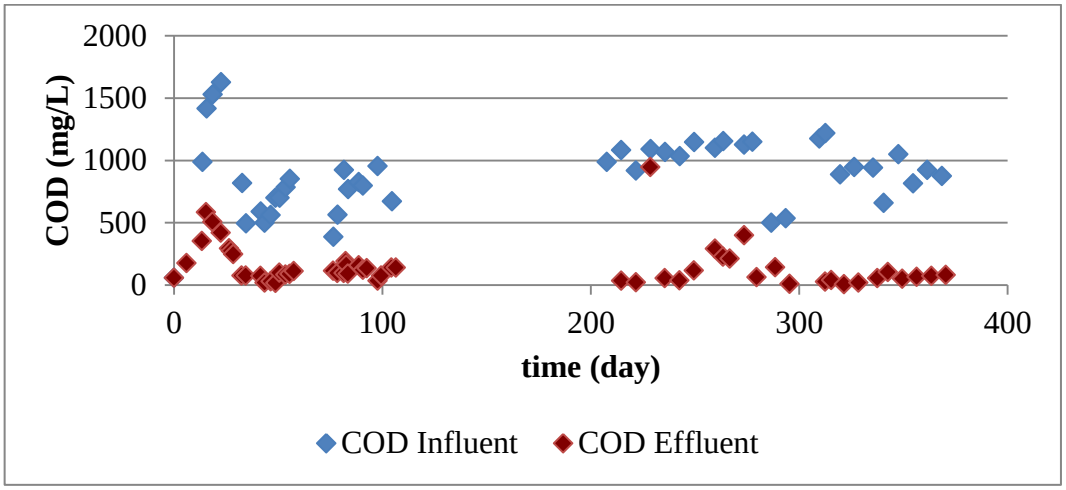


Figure 4.3. COD profiles in Control Reactor (CR)

Reactor 2 (R2) was fed with Feed 2 which had a lower organic content compared with Feed 1. As shown in Figure 4.4, R2 had lower influent COD value with respect to R1 and CR. However, all three reactors were fed at the same TKN strength. Only the change in the COD strength of the wastewater led to a change in the C/N ratio. Because organic carbon loading to R2 was lower than R1 and CR, it was expected that the activity of nitrifying organisms was higher in this reactor. Organic carbon removal in R2 reached also steady-state conditions as shown in Figure 4.4.

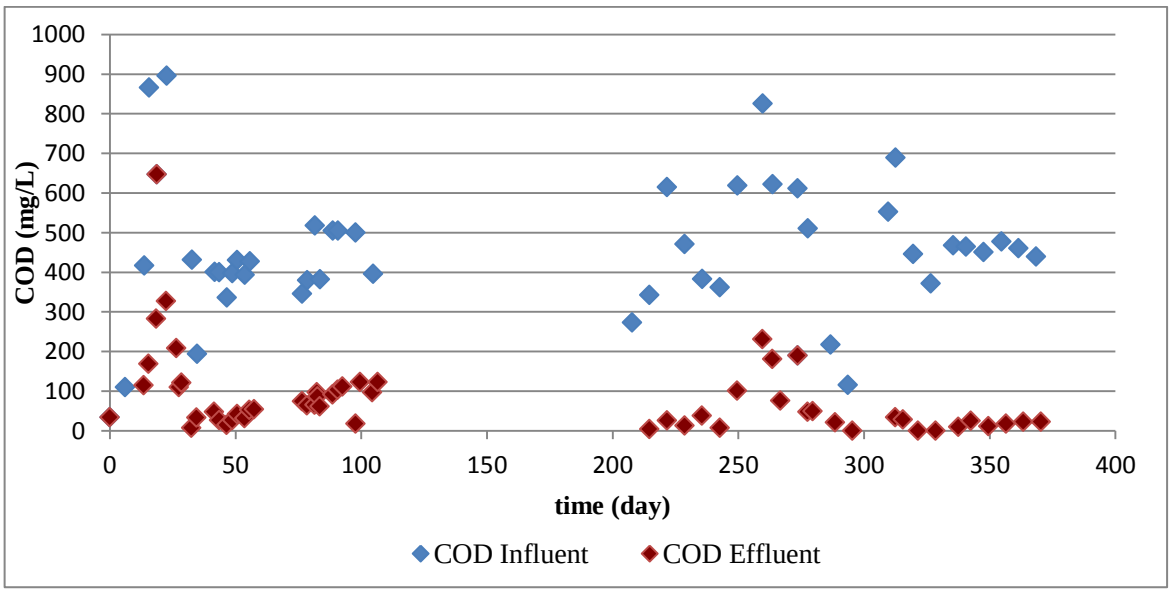


Figure 4.4. COD Profiles in Reactor 2 (R2)

4.1.2. Removal of Nitrogen in Reactors

Nitrogen removal in the reactors was monitored for the purpose of observing the differences between the reactors. Nitrogenous compounds in the reactor were removed by two phenomena; nitrification and biomass synthesis of both heterotrophic and autotrophic microorganisms. Since feeds contained mostly ammonium sulfate, the reactors monitored in terms of NH_4-N removal and TKN values were estimated.

Figure 4.5 and Figure 4.6 show the influent and the effluent NH_4-N values in R1 and CR, respectively. Influent and effluent values represent the initial and final conditions in one SCFB run (see Appendix A). As shown in Figure 4.5 and Figure 4.6, nitrogen was removed to a great extent in R1 and CR. These reactors reached steady-state conditions in terms of nitrogen removal.

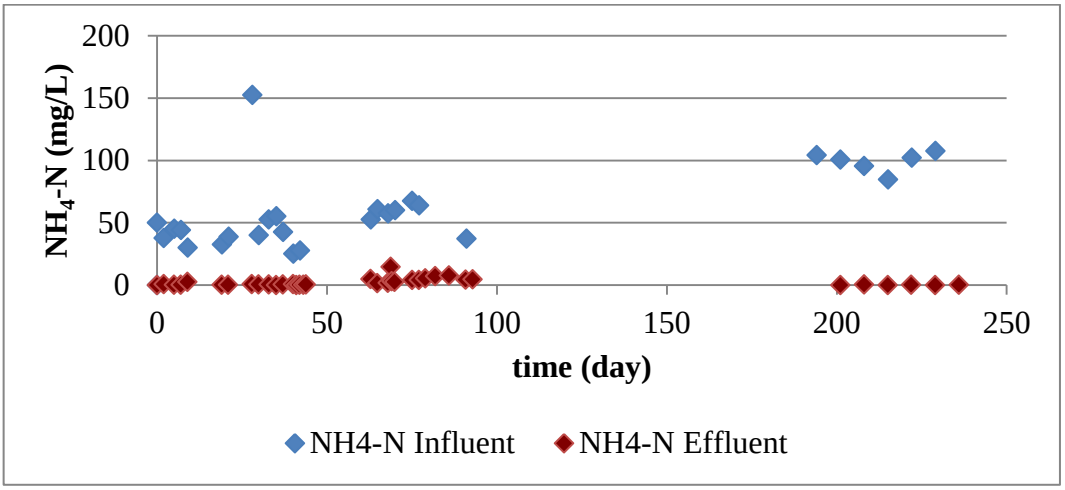


Figure 4.5. Influent and effluent NH_4-N values in Reactor 1 (R1)

Also, with regard to the C/N ratio in R1 and CR, it was expected that nitrogen removal due to nitrification is lower than other reactors. In these reactors, nitrogen is used primarily for cell synthesis rather than nitrification.

Figure 4.7 shows the influent and effluent NH_4-N in R2. Since Feed 2 had a C/N ratio of 5, it was expected that nitrification activity in this reactor was greater than R1. In this reactor, nitrogen is used up for cell synthesis and nitrification.

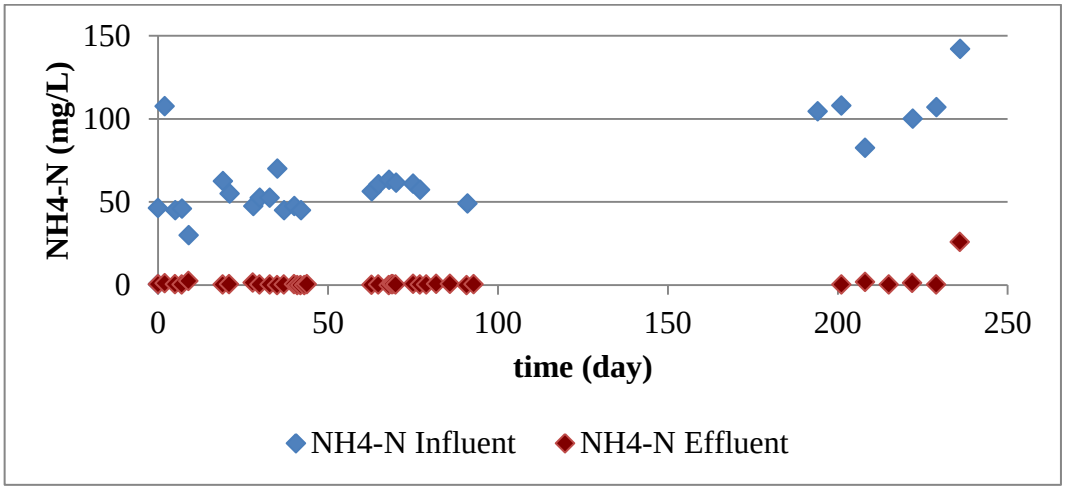


Figure 4.6. Influent and effluent NH_4-N values in Control Reactor (CR)

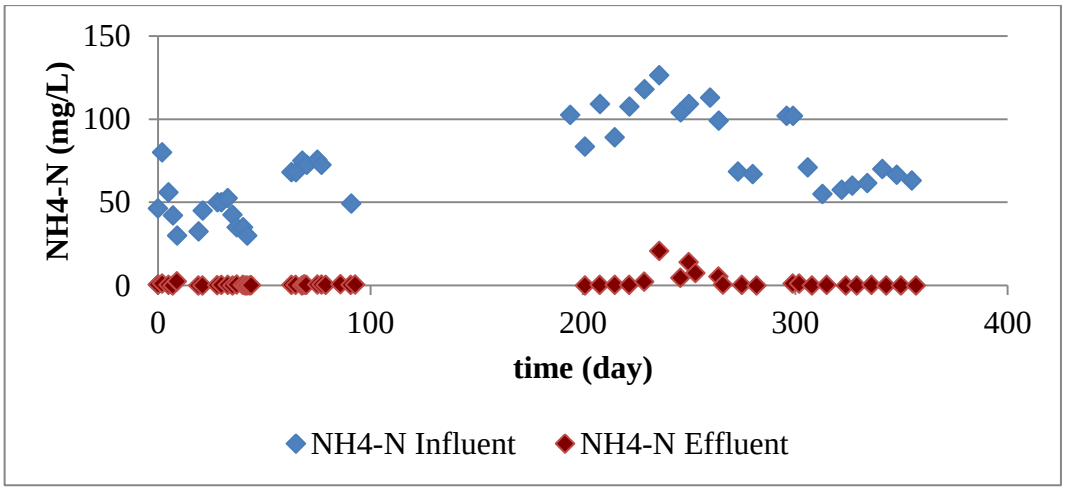


Figure 4.7. Influent and effluent NH_4-N values in Reactor 2 (R2)

Figure 4.8 shows the results belonging to R3. NH_4-N monitoring is important for R3 since it has been fed with only inorganic nitrogen. Furthermore, in order to enrich the nitrifying sludge in R3, NH_4-N loading to the reactor was increased in some periods and this caused nitrite accumulation in R3. When the data belonging to R3 in Appendix A was examined, it is seen that Reactor 3 was severely exposed to free ammonia (FA) inhibition. For some periods at high ammonium and pH values, FA (NH_3-N) concentration in Reactor 3 was calculated in accordance to Equation 2.27 which was expressed in Section 2.2.3.1 in details. Especially, FA concentrations in Reactor 3 were at maximum values in March,

2013 and reached 152.4 mg NH_3-N /L on 27th March 2013. Because of high FA concentration, nitrite accumulation occurred in R3. High NO_2-N results demonstrated that nitrite was not converted to nitrate and NOB in R3 was inhibited. Because high strength nitrogen in wastewaters, contain free ammonia and it plays decisive roles in nitrification inhibition. FA concentrations as low as 0.1-2 mg/L can cause nitrite build-up while higher concentrations cause to complete nitrification inhibition (Aktaş and Çeçen, 2001; Çeçen et al., 1995). In order to prevent FA inhibition, Reactor 3 was fed once a day since May 2013. Thus, the initial ammonium concentration was decreased.

On the other hand, free nitrous acid (FNA) concentrations in Reactor 3 were calculated in accordance with NO_2-N measurements and it was observed that FNA concentrations in R3 were in the range of 0-0.3 mg/L, mostly below the inhibitory concentration range of 0.22-2.8 mg/L (Anthonisen et al., 1976).

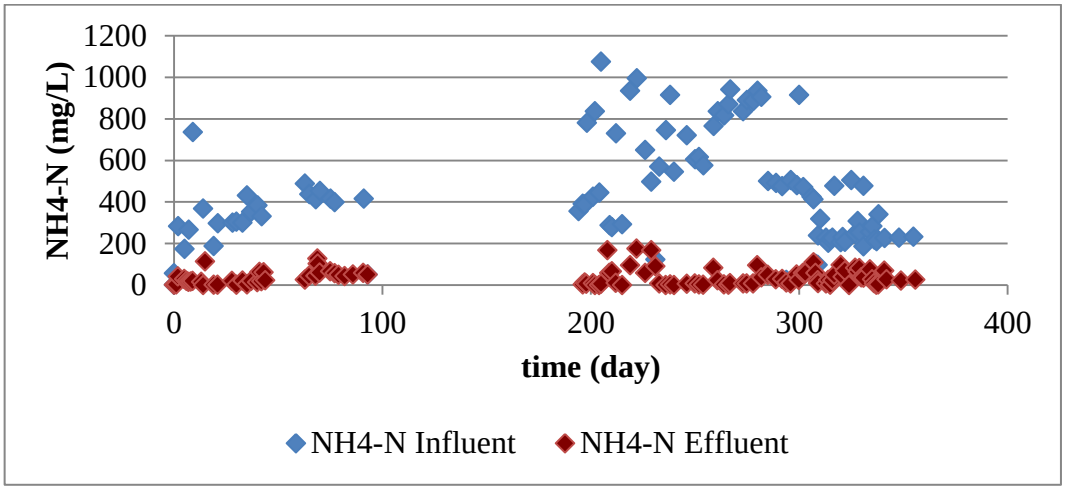


Figure 4.8. Influent and effluent NH_4-N in Reactor 3 (R3)

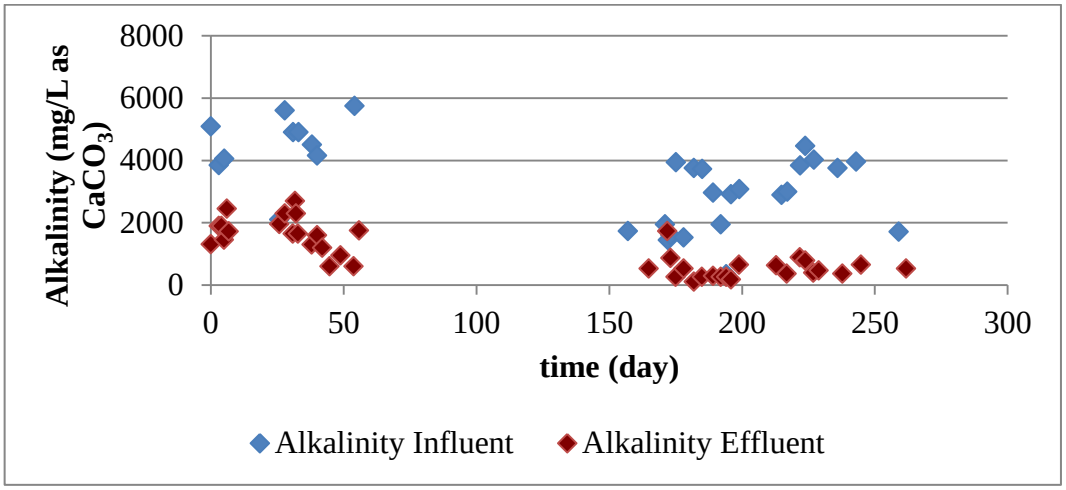


Figure 4.9. Alkalinity values in the influent and effluent of Reactor 3 (R3)

Additionally, alkalinity analyses were done in Reactor 3 in order to check the alkalinity consumption due to nitrification. Figure 4.9 shows the initial and final alkalinity values of SCFB runs for Reactor 3. Also, results for alkalinity consumption in Reactor 3 are tabulated in Appendix A. While theoretical alkalinity consumption per consumed NH_4-N is 7.05 g as $CaCO_3$/g NH_4-N (Rittman and McCarty, 2001), in our experiments the maximum value was about 7 g as $CaCO_3$/g NH_4-N in Reactor 3.

4.1.3. pH Profiles of Reactors

pH measurements were done at the beginning of feeding period in order to control the reactors. Additionally, while measuring pH, temperature measurements were done in reactors and all reactors were kept around 25 °C throughout the study. The pH values in these reactors are shown in the following figures.

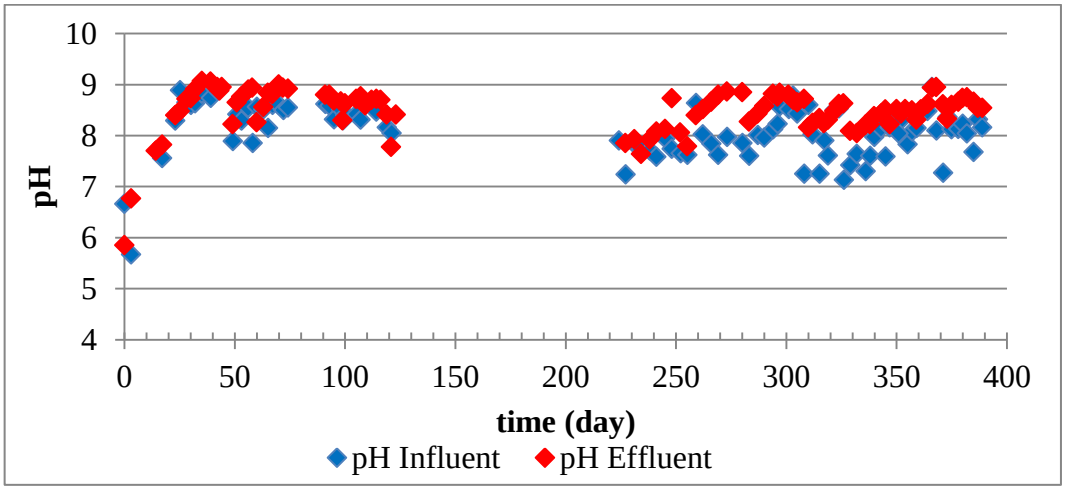


Figure 4.10. pH values under initial and final conditions of Reactor 1 (R1)

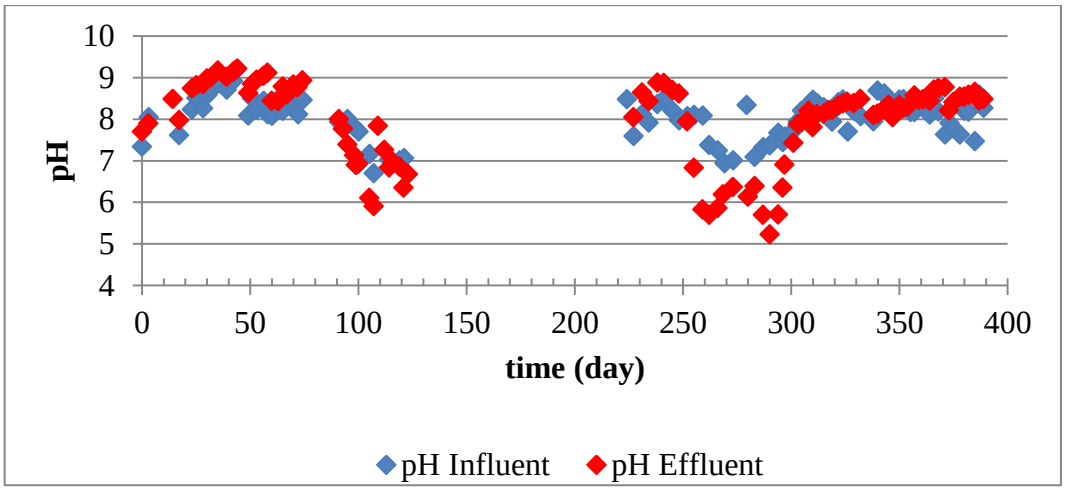


Figure 4.11. pH values under initial and final conditions of Reactor 2 (R2)

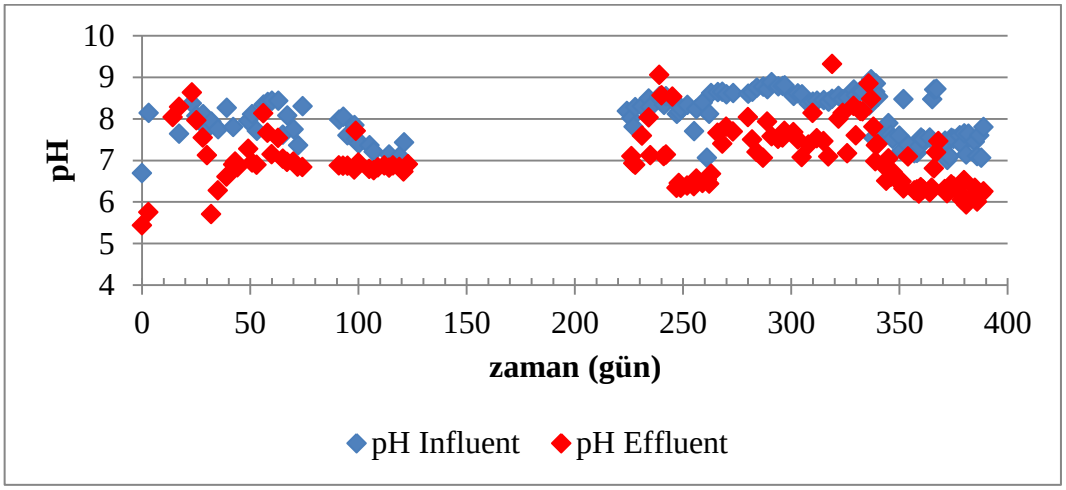


Figure 4.12. pH values under initial and final conditions of Reactor 3 (R3)

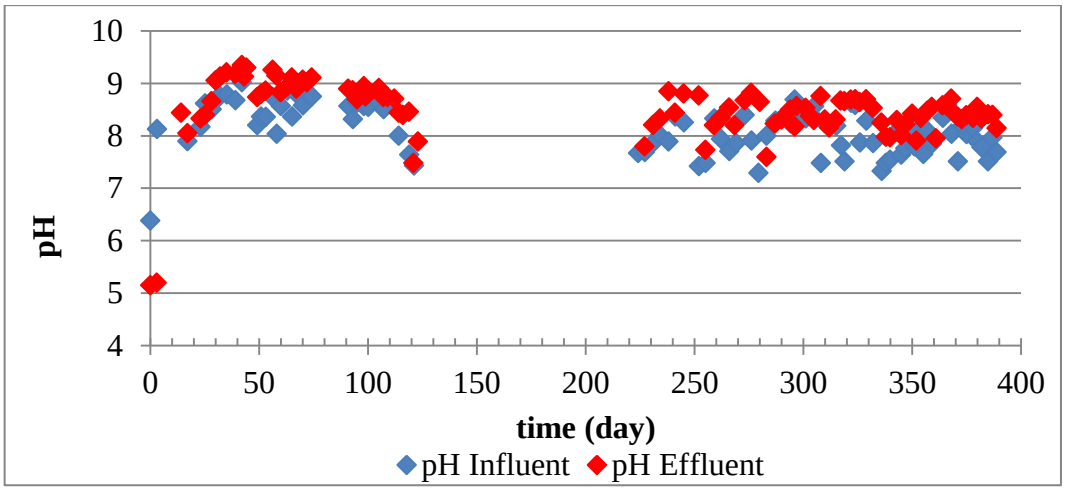


Figure 4.13. pH values under initial and final conditions of Control Reactor (CR)

4.1.4. MLSS and MLVSS Profiles in Reactors

MLSS and MLVSS analyses were done throughout the study in order to get information about the biomass concentration in the reactors. Since regularly activated sludge samples were taken from reactors for respirometry tests and EPS extraction, it is important to control the biomass level. The following figures demonstrate the MLSS and MLVSS values in these reactors.

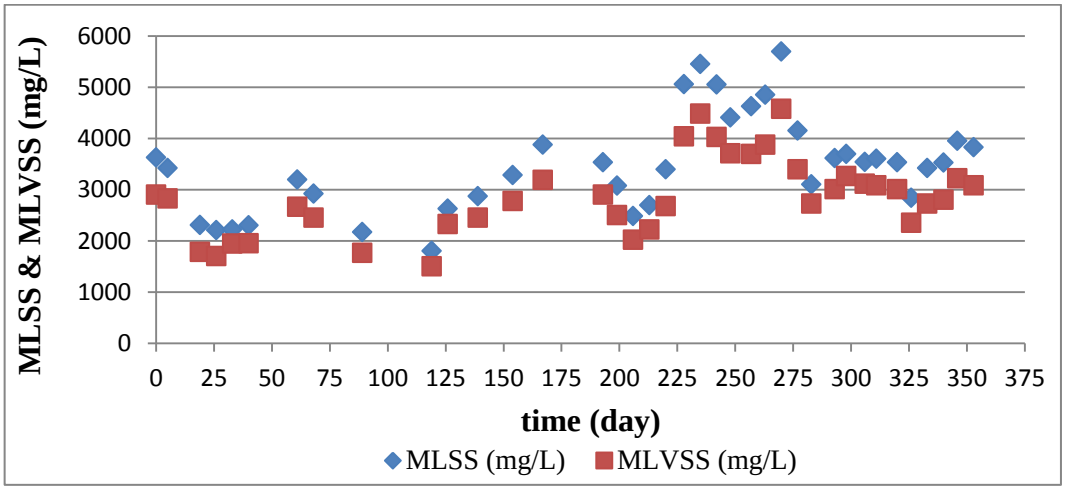


Figure 4.14. MLSS and MLVSS values in Reactor 1 (R1)

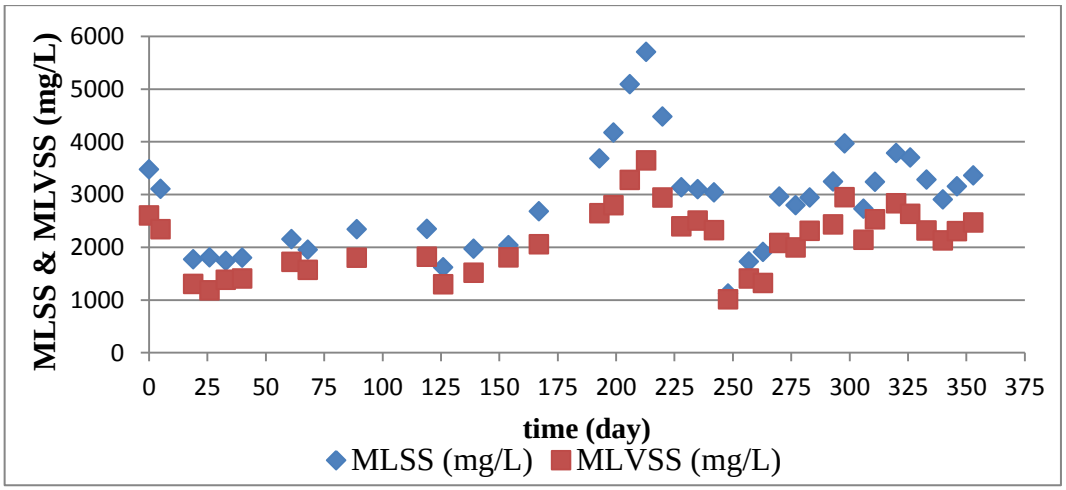


Figure 4.15. MLSS and MLVSS values in Reactor 2 (R2)

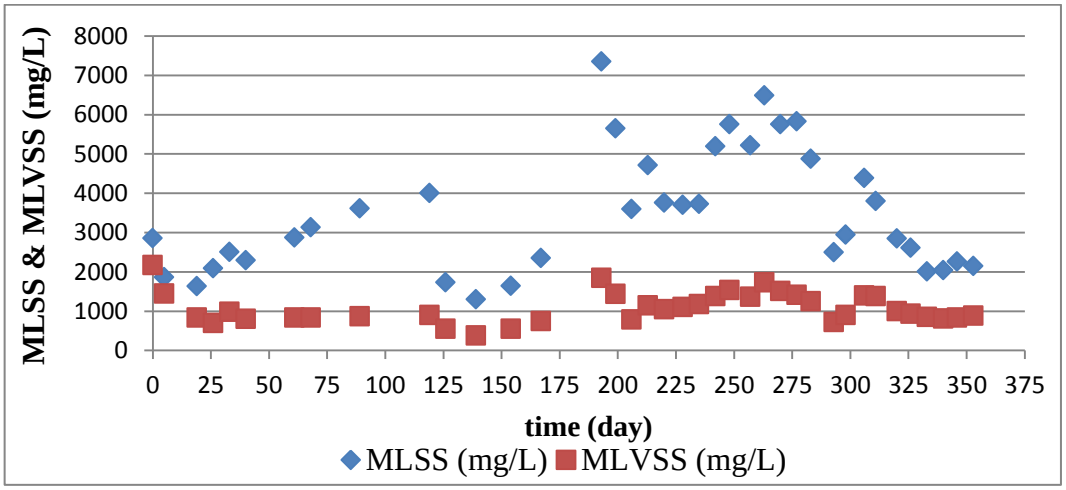


Figure 4.16. MLSS and MLVSS values in Reactor 3 (R3)

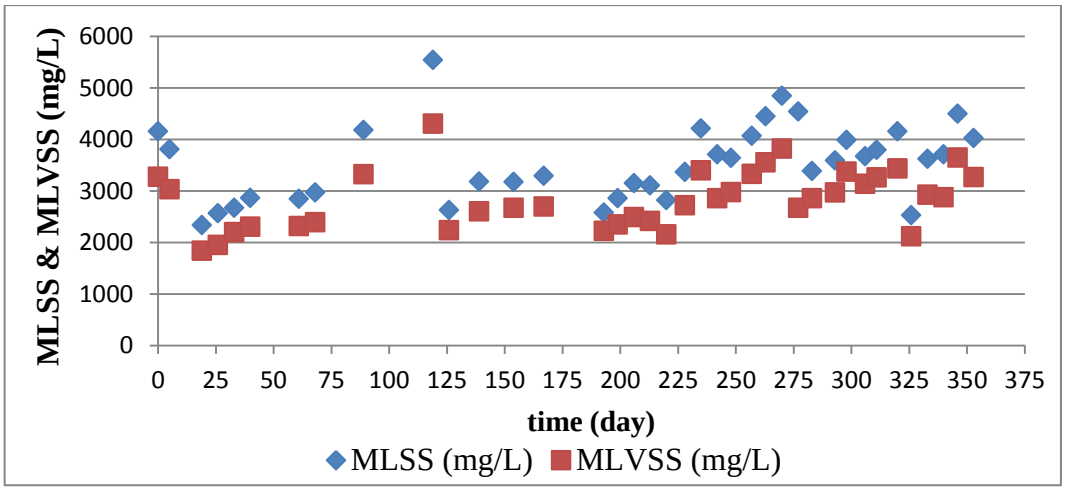


Figure 4.17. MLSS and MLVSS values of Control Reactor (CR)

Additionally, the initial substrate per biomass (S_0/X_0 ratio) in semi-continuously fed batch reactors were calculated at time zero. For this purpose MLVSS, substrate loading and duration of the periods were used. The calculations are presented in Appendix A in a tabulated form. For example, when the initial concentration in the SCFB reactor was 1000 mg/L COD and initial biomass concentration in the reactor was 2905 mg MLVSS/L, the S_0/X_0 ratio was calculated as follows:

$$\frac{S_0}{X_0} = \frac{1000 \text{ mg COD/L}}{2905 \text{ mg MLVSS/L}} = 0.34 \text{ mg COD/mg MLVSS} \quad (4.1)$$

The F/M ratio was also calculated. For this purpose, the concentration of feed stock, concentration of microorganism in the reactor and run duration was used. As an example, the F/M ratio in R1 was calculated as follows:

Substrate in concentrated feed, S= 10000 mg COD/L
Flow rate to the SCFB, Q= 400 mL/1.75 day = 0.23 L/day
The duration of a run in SCFB=1.75 day
Biomass concentration in the reactor, X= 2905 mg MLVSS/L
Volume of reactor, V= 4 L

$$\text{F: M ratio} = \frac{Q \times S}{V \times X} = \frac{0.23\text{L/day} \times 10000 \text{ mg COD/L}}{4 \text{ L} \times 2905 \text{ mg MLVSS/L}} = 0.20 \text{ mg COD/mg MLVSS. day} \quad (4.2)$$

4.2. Respirometry Tests

The respirometry tests constitute the major part of this study. The respirometric measurements can provide much information about the operation of an activated sludge. In this study, respirometry was used for two main purposes. First aim was to distinguish the organic carbon removal and nitrification and the second was to determine the inhibitory effect of the Ag on different types of activated sludge. The measurement principle of respirometry, respirometric test procedure and evaluation of raw respirometric data were presented in details in Section 3.3.2.

In Table 4.1, respirometric tests were listed in chronological order. The respirometric tests started on 18th June 2012 and ended on 17th April 2013. “Theoretical values” represent the prepared concentrations and were calculated using the substrate addition. Analytical values are found as a result of analyses.

Table 4.1. Properties of respirometric tests in chronological order

Test No:	Date	Reactor	Feed No:	THEORETICAL VALUES					ANALYTICAL VALUES			
				Feed Concentration		Ag (mg/L)	ATU (mg/L)	TCMP (mg/L)	Initial NH_4-N (mg/L)	Initial COD (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
				COD (mg/L)	TKN (mg/L)							
1	**18.06.2012**	CR	1	500	50	10 -20 - 40	0		not measured	432	2510	2172
2	**02.07.2012**	R3	3	-	50	0.5 - 1	11.6		41	0	528	474
3	**05.07.2012**	R1 & R2	1	500	50	5	11.6		not measured	494	628	520
			2	250	50				not measured	497	690	573
4	**10.07.2012**	R1	1	500	50	5 - 10	11.6		not measured	480	755	714
5	**18.10.2012**	CR	1	500	50	5 - 10		10	29	432	948	864
6	**22.10.2012**	CR	1	500	50	5 - 10		10	37.75	414	1588	1292
7	**08.11.2012**	R1	1	500	50	1 - 5		10	32	not measured	1715	1540
8	**19.11.2012**	R3	3	-	50		11.6		35.5	not measured	336	172
9	**22.11.2012**	R1	1	500	50		11.6		31	457	1066	969
10	**26.11.2012**	R2	2	250	50		11.6		34.75	190	640	584
11	**06.12.2012**	R1	1	500	50	2	11.6		26.75	351	1692	1414
12	**15.01.2013**	R1	1	500	50	10 - 20 - 40 - 50			32.75	332	1270	1210
13	**17.01.2013**	R1	1	500	50	5	11.6 (Pre.*)		27.5	357	760	713

Table 4.2. Properties of Respirometric tests in chronological order (continued)

Test No:	Date	Reactor	Feed No:	THEORETICAL VALUES					ANALYTICAL VALUES			
				Feed Concentration		Ag (mg/L)	ATU (mg/L)	TCMP (mg/L)	Initial NH_4-N (mg/L)	Initial COD (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
				COD (mg/L)	TKN (mg/L)							
14	**24.01.2013**	R1	1	500	50	1 - 2- 4- 5			37	497	1245	1125
15	**31.01.2013**	R1	1	500	50	1 - 2 - 4 - 5			38	378	1950	1765
16	**06.02.2013**	R2	2	250	50	0.5 - 1 - 1.5 - 3			31.25	131	1215	1045
17	**14.02.2013**	R2	2	250	50	1 - 1.5 - 2	11.6 (Pre.*)		35	156	1350	1165
18	**27.02.2013**	R1	1	500	50	2	11.6 (Pre.*+ direct**)		39	504	1425	1335
19	**06.03.2013**	R2	2	250	50	2	11.6 (Pre.*+ direct**)		37	192	1155	970
20	**13.03.2013**	R1	1	500	50				34.25	462	1568	1392
21	**14.03.2013**	R3	3	-	50				43.25		3345	1025
22	**21.03.2013**	R2	2	250	50				43.75	214	1200	1085
23	**01.04.2013**	R1	1	500	50	2 -3 - 4		10.6	34.25	479	1660	1540
24	**09.04.2013**	R1	1	500	50		11.6	10.6 - 21.4 - 31.8	39	501	840	689
25	**16.04.2013**	R3	3	-	50		11.6	10.6 - 21.4 - 31.8	50		1305	1135
26	**17.04.2013**	R2	2	250	50		11.6	10.6 - 21.4 - 31.8	36	213	1585	1280

*Pre: Previously ATU contact. Activated sludge was aerated with ATU before the respirometry test.
**Direct: Directly ATU contact. ATU was added to respirometric chambers at the start of respirometry test.

Analytical measurements were done under the initial and final conditions of respirometric chambers. The purpose of doing analytical measurements is to compare with respirometric results. As shown in Figure 4.18, three unknowns can be calculated by respirometry tests and analytical measurements. The first one is O_2 uptake and CO_2 production and is labelled as 1. They were measured by respirometer. COD, NH_4-N (substrate) were found analytically and are labelled as 2. Biomass formation is estimated by using the biokinetic constants in literature and is labelled as 3.

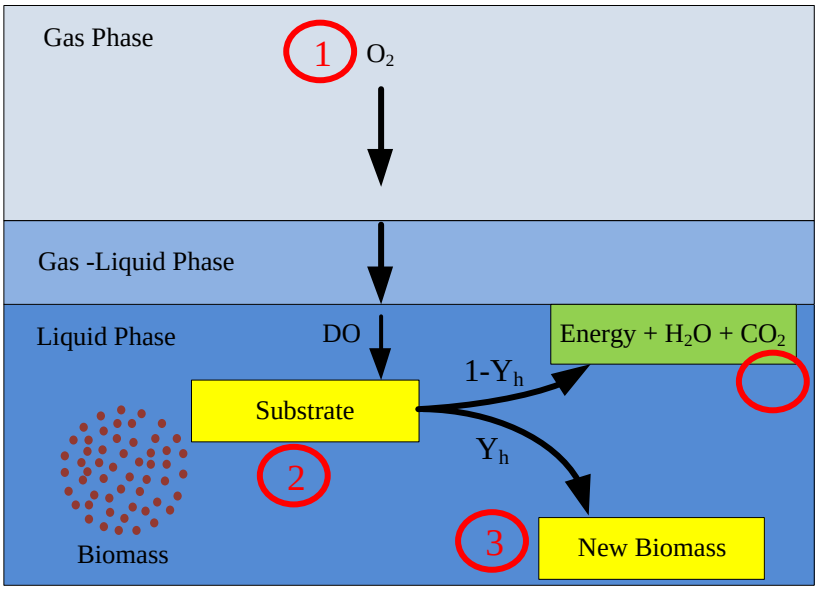


Figure 4.18. Relationship between oxygen utilization and biomass growth

As shown in Figure 4.18, microorganisms use substrate as an energy source and for the formation of new cells simultaneously. When microorganisms use substrate, some portion is used for respiration and the other portion is used for forming new cells. As an example, organic substrate is equivalent to 1 COD unit, Y_h portion of it is used in cell synthesis and (1- Y_h) portion is used for respiration (energy production). As mentioned in "Literature Review", Y_h value is represented in units of cell COD/removed substrate COD and is in the range of 0.6-0.69 (Rittman and McCarty, 2001).

Therefore, when organic matter is removed, the oxygen consumption shows the fraction of COD which is used in energy reactions. Thus oxygen consumption is related to Y_h value which can be estimated from respirometry tests (Vanrolleghem et al., 1999; Vanrolleghem, 2002; Vanrolleghem et al., 2003)

In addition to that, theoretical oxygen consumption for complete oxidation of 1 mg NH_4-N to NO_3-N is 4.33 mg O_2. In this oxygen consumption, 3.22 mg O_2 is used in converting NH_4-N to NO_2-N and 1.11 mg O_2 in converting NO_2-N to NO_3-N. In a nitrifying system, a large portion of ammonium (95-98%) is oxidized to nitrite and nitrate and a small portion of ammonium is used in cell synthesis. Therefore, the yield coefficient (Y_a) of nitrifying bacteria is very low when compared with heterotrophic bacteria (Grady et al., 2011). Since Y_a value of nitrifying bacteria is very low, in nitrification the ammonium used for cell synthesis can be neglected.

Therefore, according to analytical measurements of respirometry tests, N-O_2 consumption can be calculated and checked with respirometric readings. For instance, if in a respirometry test, NH_4-N removal due to nitrification is found as 24.67 mg NH_4-N/L by analysis, the cumulative N-O_2 consumed in the gas phase should be theoretically 10.68 mg O_2 as follows:

$$24.67\ \text{mg}\ NH_4^+ - N/L \times 4.33\ \text{mg}\ O_2/\text{mg}\ NH_4^+ - N \times 0.1\ \text{L sample volume} = 10.68\ \text{mg}\ O_2 \quad (4.3)$$

In this way, respirometric N-O_2 data can be compared with analytical results. Also, analytical results can be checked with respirometric data.

4.2.1. Determination of the Sensitivity of Respirometric Data

These tests were conducted in order to investigate the reliability of respirometric tests. Errors that occurred during sample preparation and fluctuations in respirometric measurements can cause differences between chambers. Therefore, **Respirometric Test 20, 21 and 22** were done for observing these differences. R1, R3 and R2 sludge were used in **Respirometric Test 20, 21 and 22**, respectively. The procedure in these tests was

explained in “Materials and Methods”. Because all chambers contained identical samples, it was expected that all measurements in one interval were close to each other. The average values of each interval were calculated from raw respirometric data and 95 % confidence interval was found.

Confidence interval is a statistical interval estimation which is conducted in a normal distribution whose expected value and standard deviation is μ and σ, respectively. A confidence interval is always calculated by first choosing a confidence level. Confidence level shows the degree of the reliability of an interval. If the confidence level is selected as 95 % for the confidence interval determination of n results, it is expected that 95 % of n results is in the range of upper and lower limit of the interval (Devore, 2008).

In order to calculate the confidence interval, firstly, experimental results are standardized and fit to a standard normal distribution. It is assumed that Z is the standardized random variable of X. X can be standardized by subtracting its expected value and then dividing by its standard deviation as follows:

$$Z = \frac{X - \mu}{\sigma/\sqrt{n}} \tag{4.4}$$

Z values of random variable X forms the standard normal distribution curve. Area that is under the standard normal distribution curve between -1.96 and +1.96, demonstrates the 95 % of whole area. In accordance to this information, 95 % confidence interval is calculated by the assumption that Z value is in the range of -1.96 and +1.96 with the probability of 95 %. This is expressed as follows:

$$P\left(-1.96 < \frac{X - \mu}{\sigma/\sqrt{n}} < +1.96\right) = 0.95 \tag{4.5}$$

When the inequality in Equation 4.5 is solved, it is found that the expected value (μ) is between these two values with possibility of 95 %.

$$\left(X - 1.96\frac{\sigma}{\sqrt{n}} < \mu < X + 1.96\frac{\sigma}{\sqrt{n}}\right) = 0.95 \quad (4.6)$$

These end points for μ are random because end points involve a random variable, X. However, confidence interval is not random because it is centered at X and extends to 1.96*σ/√n on each side of X with respect to standard deviation, σ and sample number, n. Thus the interval width is 2*(1.96)* σ/√n (Devore, 2008).

In respirometry tests, values recorded by the respirometer are expected to be in a specific interval, this interval shows that 95 % of the data are in this boundary and the width of interval determines the closeness of data. In these tests, upper and lower limits for each interval were calculated in a 95 % confidence interval. Figure 4.19, Figure 4.20and Figure 4.21 represent the average of respirometric results obtained with R1, R2 and R3 sludges and upper and lower limits in 95 % confidence interval. As shown in figures, CO_2 measurements fluctuated less with respect to O_2 measurements.

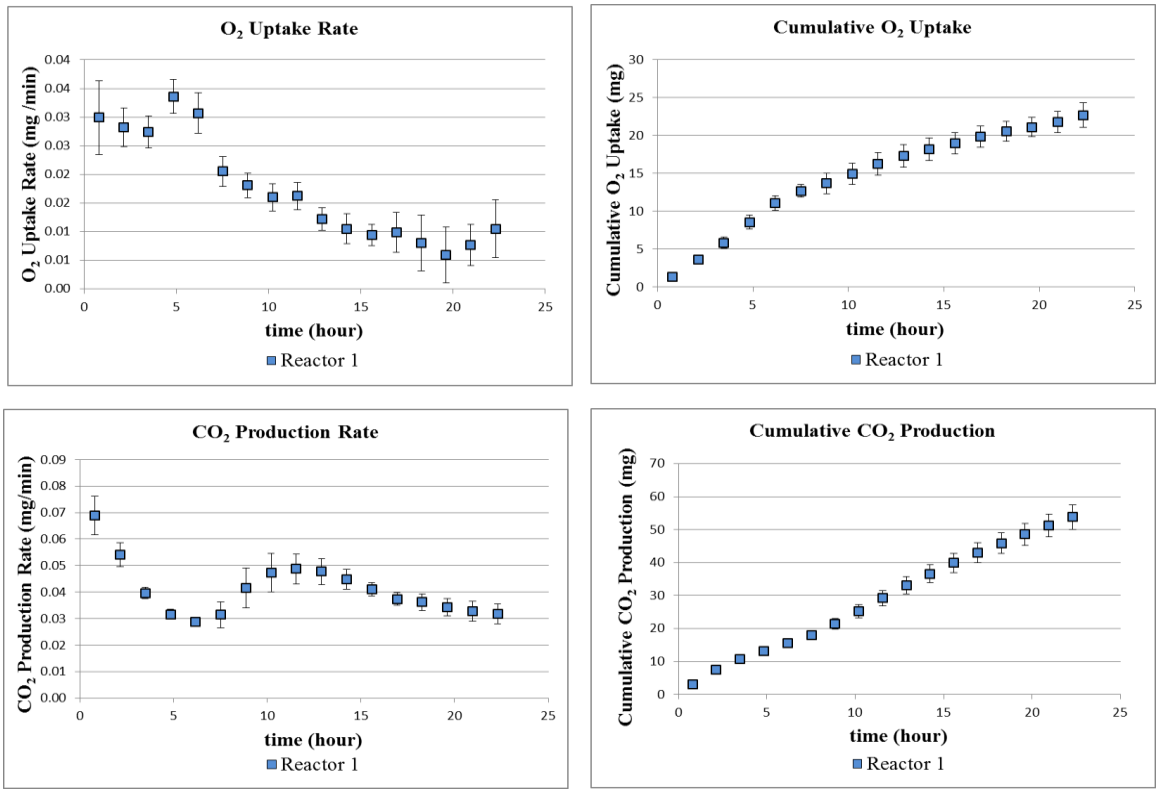


Figure 4.19. Average values and 95 % confidence interval of respirometric data in Reactor 1 (Test 20)

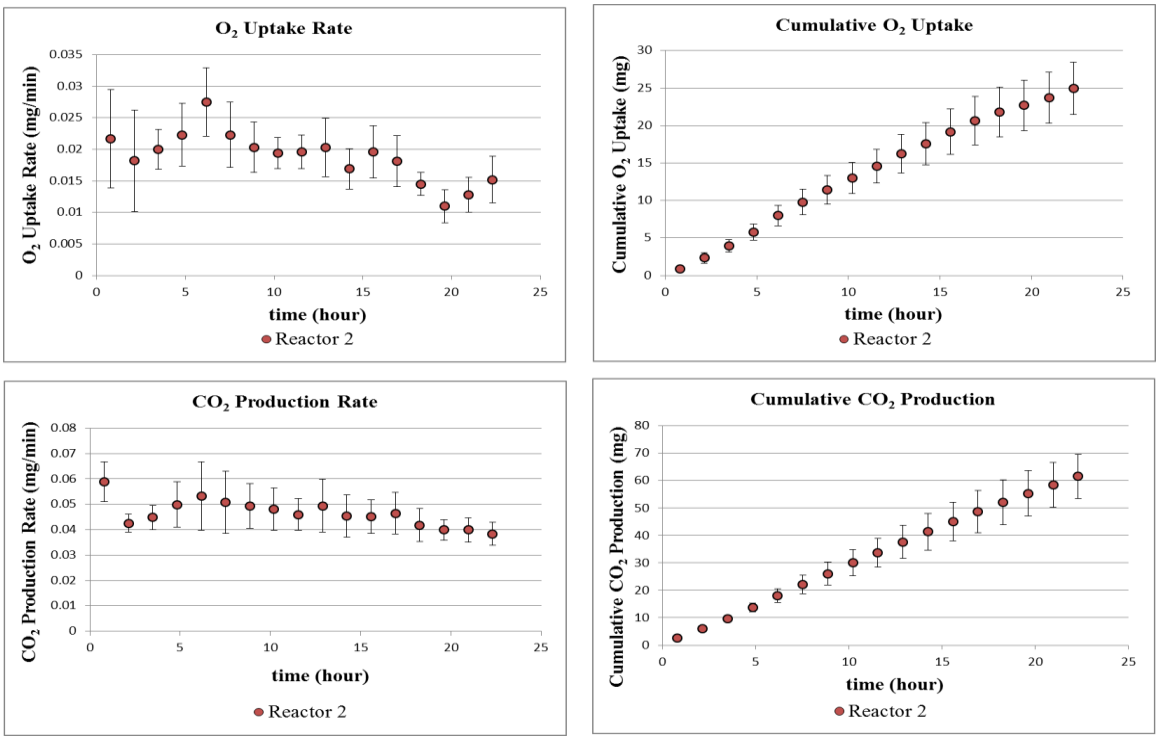


Figure 4.20. Average values and 95 % confidence interval of respirometric data in Reactor 2 (Test 22)

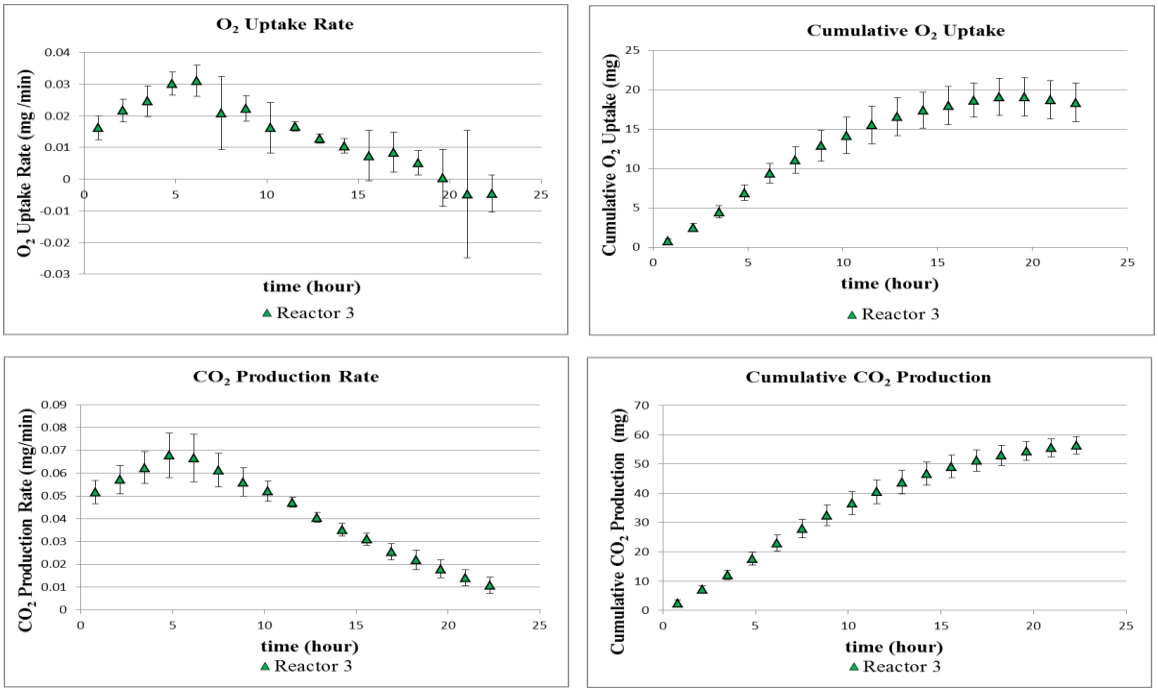


Figure 4.21. Average values and 95 % confidence interval of respirometric data in Reactor 3 (Test 21)

These tests provided us to make better interpretation from respirometric results. Very close values in respirometric results can cause confusion about that it is equal or not. On the other hand, One reading is in the range of error margin of other reading, it can be assumed as same value in 95 % confidence level. In these tests, for each measurement, margin of error were found in 95 % confidence interval.

4.2.2. Determination of Organic Carbon Removal and Nitrification

In order to observe carbonaceous oxygen uptake (C-O_2) and nitrogenous oxygen uptake (N-O_2) of reactors, respirometry tests were done by using a nitrification inhibitor. Procedures were given in detail in "Materials and Methods".

4.2.2.1. Results of Reactor 1 (C/N Ratio: 10). **Respirometric Test 9, 13, 18** and **24** were done in order to distinguish carbonaceous oxygen uptake and nitrogenous oxygen uptake in R1 sludge.

Respirometric Test 9 was done on 22th November 2012. In this test, ATU was used as a nitrification inhibitor. The MLSS and MLVSS of test samples were 1066 and 969 mg/L, respectively. Figure 4.22 shows the cumulative oxygen consumption in terms of total oxygen uptake (T-O_2), carbonaceous oxygen uptake (C-O_2) and nitrogenous oxygen uptake (N-O_2). According to figure, heterotrophic activity is much greater than autotrophic activity. The oxygen consumption due to organic carbon removal (T-O_2) is seen as 34 mg O_2 while it is 6 mg O_2 due to (N-O_2) nitrification. Analytical results showed that 18.67 mg/L NH_4-N was used in nitrification reactions and 9.67 mg/L NH_4-N was used for heterotrophic cell synthesis. However, nitrification was not complete and nitrite accumulation occurred in test chambers. According to analytical results, approximately 9 mg/L N was converted to NO_3-N in test chamber (No. 4. 5. 6.).

When ammonium is converted to nitrite in nitrification, using the analytical results, oxygen consumption due to nitrification can be calculated as follows:

$$18.67\ \text{mg}\ NH_4 - N/L \times 3.22\ \text{mg}\ O_2/\text{mg}\ NO_2 - N \times 0.1\ L = 6.01\ \text{mg}\ O_2 \qquad (4.7)$$

This calculated value also corresponds to respirometric N-O_2 result of 6 mg O_2. In Figure 4.22 and Figure 4.23, N-O_2 values are seen as negative until the 15th hour. The reason of that, carbonaceous oxygen consumption until 15th hour nearly equal to T-O_2, and this cause the negative results in N-O_2. In this time period, N-O_2 was at minimal value and respirometric measurements could not detect it. However, negative results cannot exist and they should be taken as zero in reality.

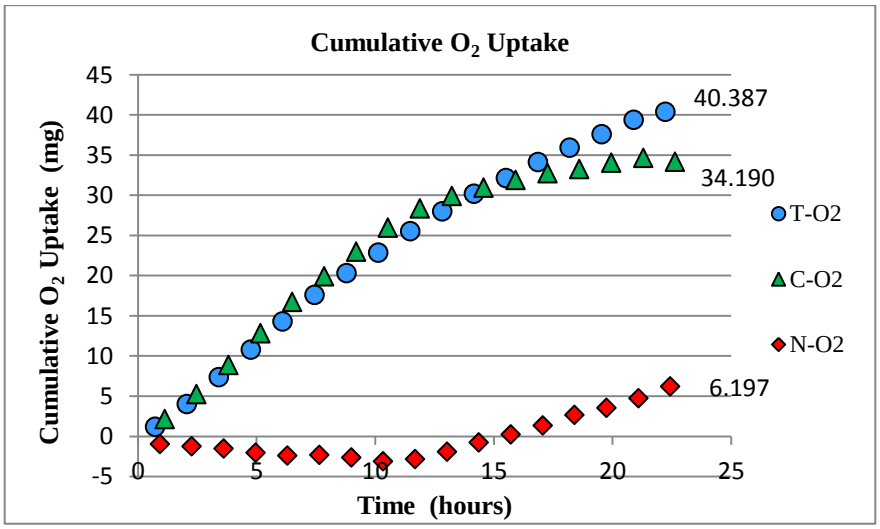


Figure 4.22. T-O_2, N-O_2 and C-O_2 results in Respirometric Test 9 (R1-22.11.2012)

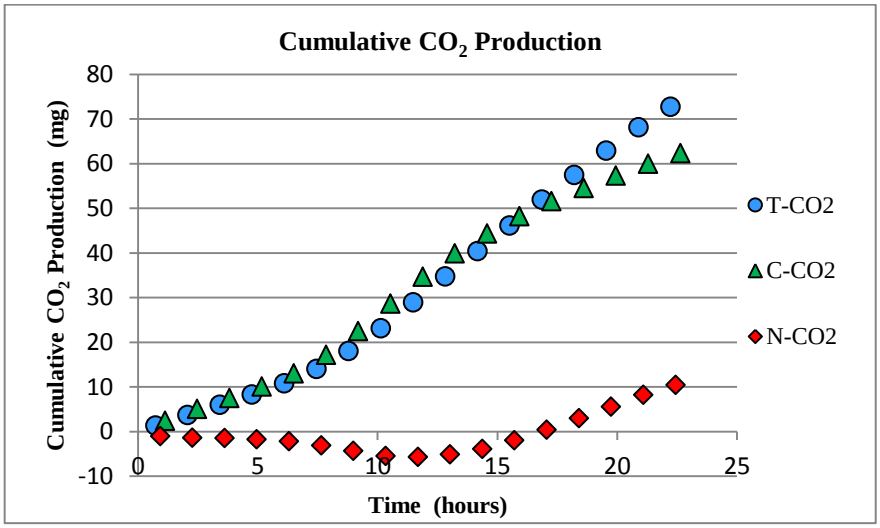


Figure 4.23. T-CO_2, N-CO_2 and C-CO_2 results in Respirometric Test 9 (R1-22.11.2012)

In **Respirometric Test 13 and 18**, activated sludge samples taken from R1 were aerated with ATU for approximately 15 hours. Then, activated sludge samples were washed with deionized water before tests in order to remove ATU from medium. Also, for comparison purposes, one portion of activated sludge was aerated without ATU. This sludge sample was used in control chambers for observing respiration activities under normal conditions. Additionally, in **Respirometric Test 18**, ATU was added to the respirometric chambers at the beginning of the test.

C-O_2 and N-O_2 separation was done with the Pre-ATU method in **Respirometric Test 13**. As seen in Figure 4.24, N-O_2 value is very low. This indicates that organic carbon removal dominated in R1. According to analytical results, NH_4-N removal in nitrification is 2 mg/L. This amount corresponds to a low O_2 concentration of 0.9 mg O_2. Besides, respirometric results show that N-O_2 value in this experiment is 0.4 mg O_2. Figure 4.25 shows the carbon dioxide production of R1 sludge in terms of T-O_2, C-O_2 and N-O_2. However, N-O_2 and C-O_2 could not be separated in this graph because T-O_2 and C-O_2 values are so close to each other. According to **Respirometric Test 20**, T-CO_2 data can be read with ±3.7 mg CO_2 error. For this reason, it is accepted that C-CO_2 is the same as T-CO_2.

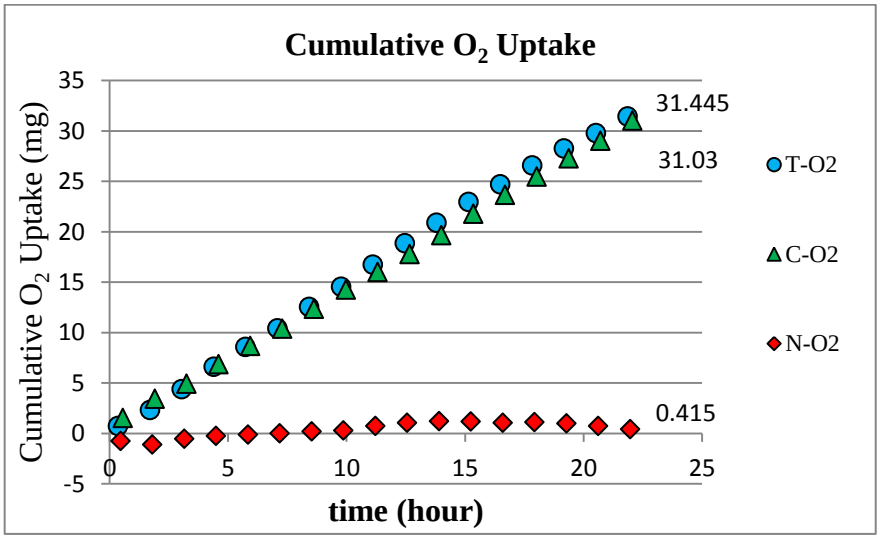


Figure 4.24. T-O_2, N-O_2 and C-O_2 results in Respirometric Test 13 (R1-17.01.2013)

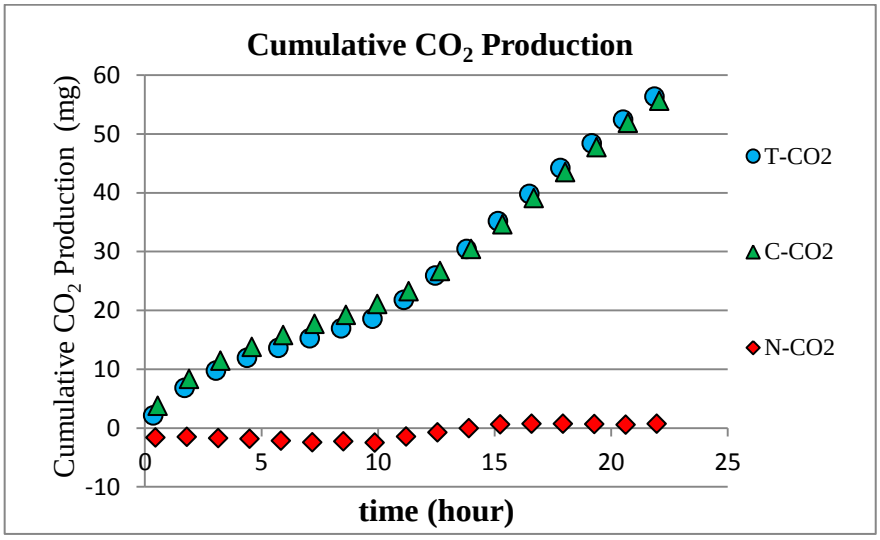


Figure 4.25. T-CO_2, N-CO_2 and C-CO_2 results in Respirometric Test 13 (R1-17.01.2013)

In **Respirometric Test 18**, Pre-ATU and direct ATU method gave similar analytical results with regard to nitrification. Therefore, it can be stated that previous and direct ATU contact influenced activated sludge to the same extent. However, as seen in Figure 4.27, oxygen consumption due to nitrification is around 2 mg O_2. When analytical results were examined, oxygen consumption due to nitrification was calculated as 3.5 mg O_2 and analytical results confirmed respirometric readings. The extent of nitrification is very small compared to organic carbon removal. NH_4-N removal in the control chamber and the sample preaerated with ATU was 30.8 and 22.8 mg/L, respectively. From this difference, the theoretical N-O_2 difference between two samples can be calculated as follows:

$$30.8 - 22.8 = 8 \text{ mg } NH_4 - N/L \tag{4.8}$$

$$8 \text{ mg } NH_4 - N/L \times 4.33 \text{ mg } O_2/g\, NH_4 - N \times 0.1 \text{ L} = 3.5 \text{ mg } O_2$$

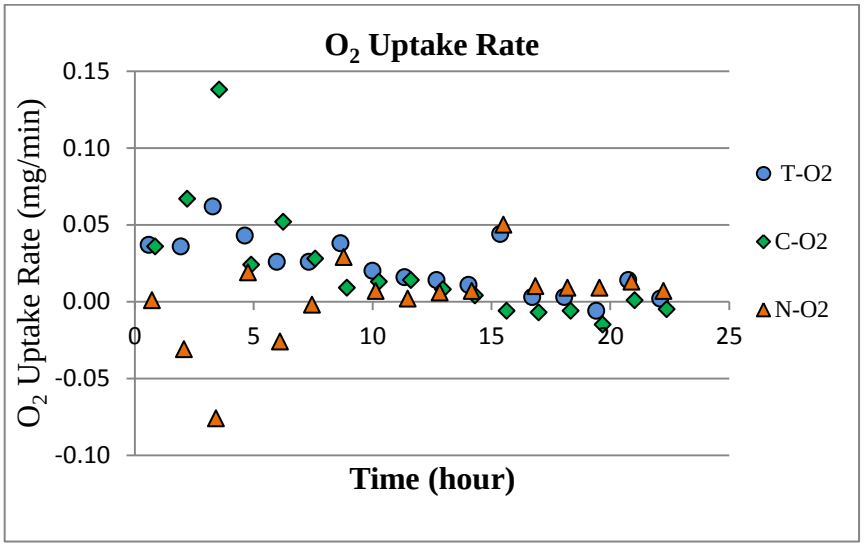


Figure 4.26. T-O_2, N-O_2 and C-O_2 uptake rate results in Respirometric Test 18 (R1-27.02.2013)

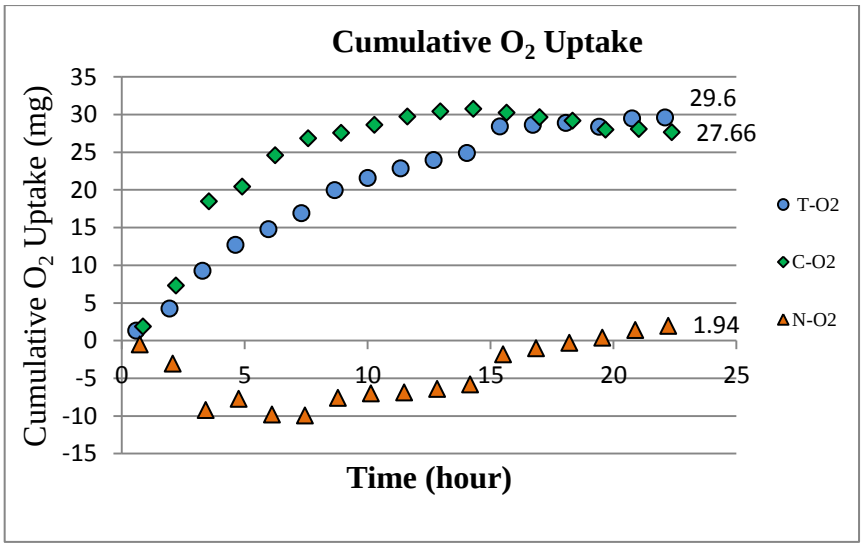


Figure 4.27. T-O_2, N-O_2 and C-O_2 results in Respirometric Test 18 (R1-27.02.2013)

In **Respirometric Test 24**, TCMP was used at various concentrations for inhibiting nitrification in R1 sludge. Also, ATU was used in order to compare the inhibitory effect. Figure 4.28 demonstrates the average cumulative oxygen consumption in various test samples. As shown in Figure 4.28, N-O_2 values cannot be separated with respirometric measurements because with or without ATU very close respiration data were obtained since the extent of nitrification was very small.

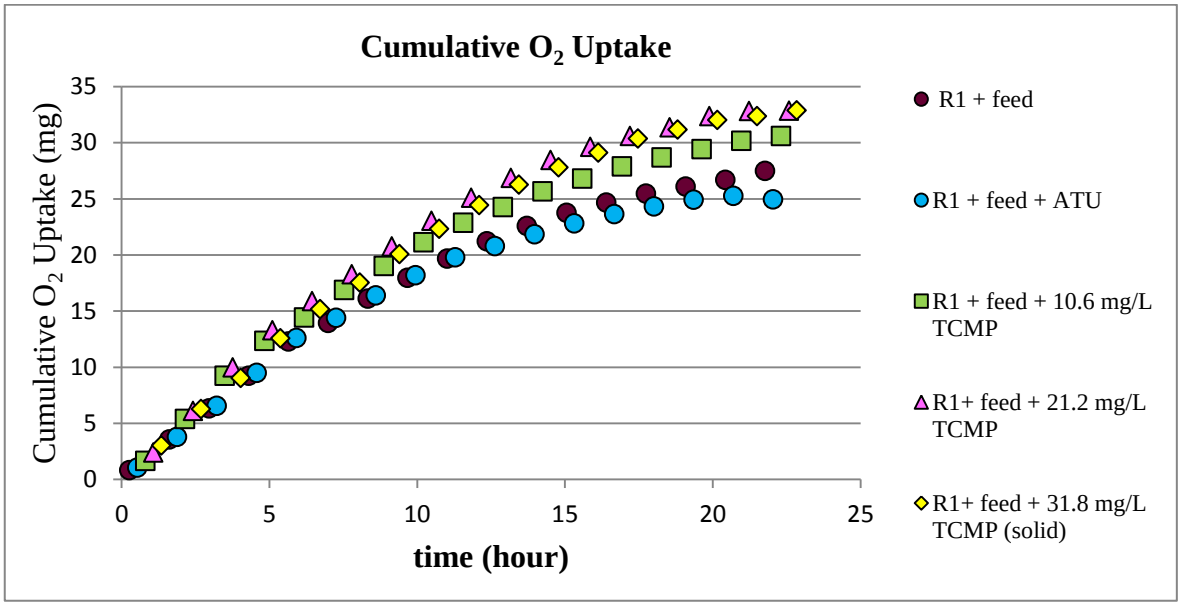


Figure 4.28. Results in Respirometric Test 24 (R1-09.04.2013)

Analytical measurements of this test show that nitrification inhibitor affected R1 sludge slightly. This sludge had primarily heterotrophic activity. When ammonium removal in Table 4.3 is examined, 21.2 mg/L and higher TCMP concentrations showed the same inhibitory effect on R1 sludge as ATU.

Table 4.3. Analytical results of Respirometric test 24

Properties of Test Chambers	Average COD Removal (mg/L)	Average NH_4-N Removal (mg/L)
Control Chamber	501	19.5
11.6 mg/L ATU	502	15.13
10.6 mg/L TCMP	510	18.25
21.2 mg/L TCMP	501	15.25
31.8 mg/L TCMP	515	15.38

In **Respirometric Test 5, 6 and 7**, pure TCMP was used for the purpose of separating C-O_2 and N-O_2. The raw results of these tests are represented in Appendix B. Since it was not obtained reliable and expected results from these tests, they were not examined in detail.

4.2.2.2. Results of Reactor 2 (C/N Ratio: 5). **Respirometric Test 10, 17, 19 and 26** were conducted in order to determine heterotrophic and autotrophic activity in R2 sludge.

In **Respirometric Test 10** the nitrification inhibitor ATU was used to inhibit nitrification and to determine autotrophic activity in the sludge. As shown in Figure 4.29, oxygen uptake rate of nitrifiers increased after 10 hours after depletion of organic substrate removal. Also, Figure 4.30 shows that cumulative N-O_2 in R2 is greater than R1 (see Figure 4.22). Depletion of organic substrate causes the decrease in carbonaceous oxygen uptake rate. With time, nitrifiers gained advantage and N-O_2 rate increased. Additionally, respirometric measurements and analytical results were in accordance with each other in terms of nitrification. According to analytical results, 26.5 mg/L N was removed and N-O_2 was calculated as 11.5 mg O_2.

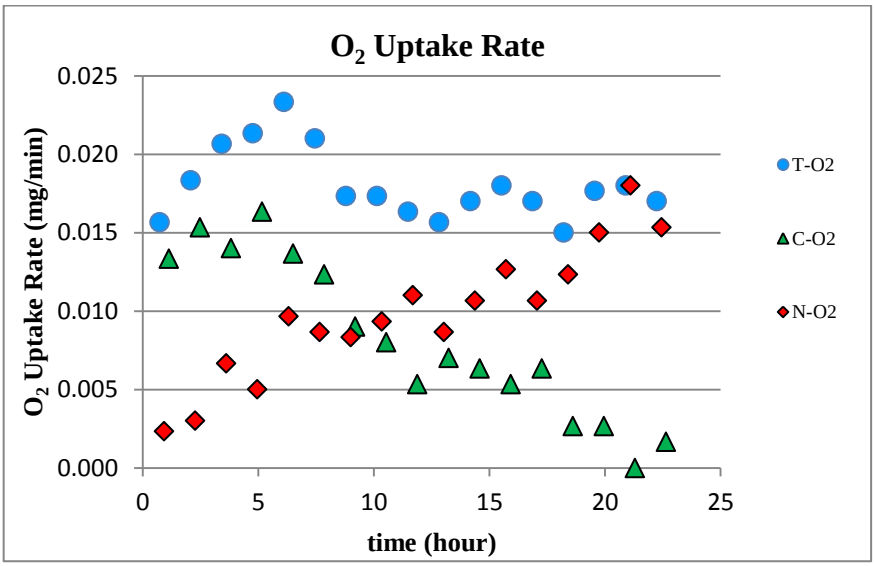


Figure 4.29. T-O_2, C-O_2 and N-O_2 uptake rate results in Respirometric Test 10 (R2-26.11.2012)

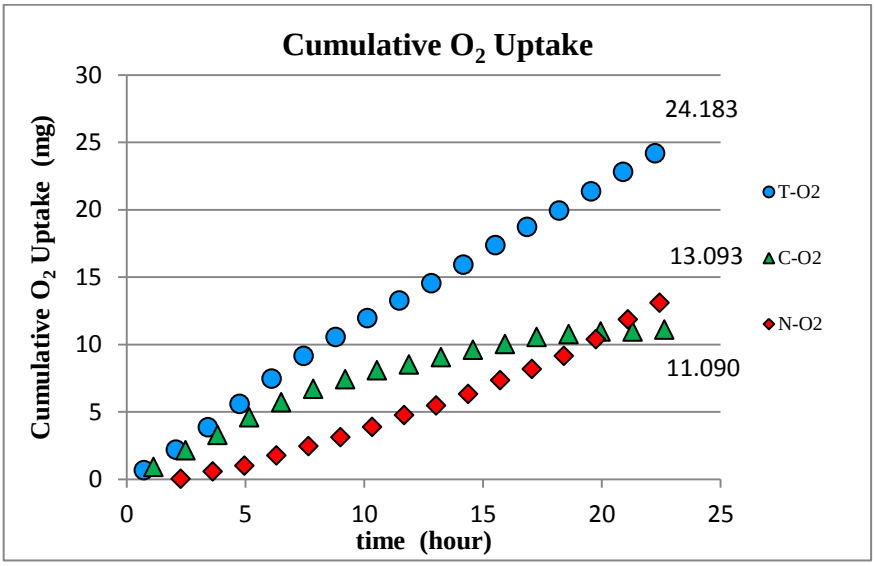


Figure 4.30. T-O_2, C-O_2 and N-O_2 results in Respirometric Test 10 (R2-26.11.2012)

Aeration of activated sludge with ATU before respirometry tests (Pre-ATU method) did not have long-lasting inhibitory effect on the nitrifying bacteria in R2 sludge. Respirometric results showed that nitrification still occurred in test samples in such cases. Figure 4.31 shows the separation of N-O_2 and C-O_2 with the Pre-ATU method. However, wrong results were obtained. It seems that major respiration activity in the system originates from heterotrophs.

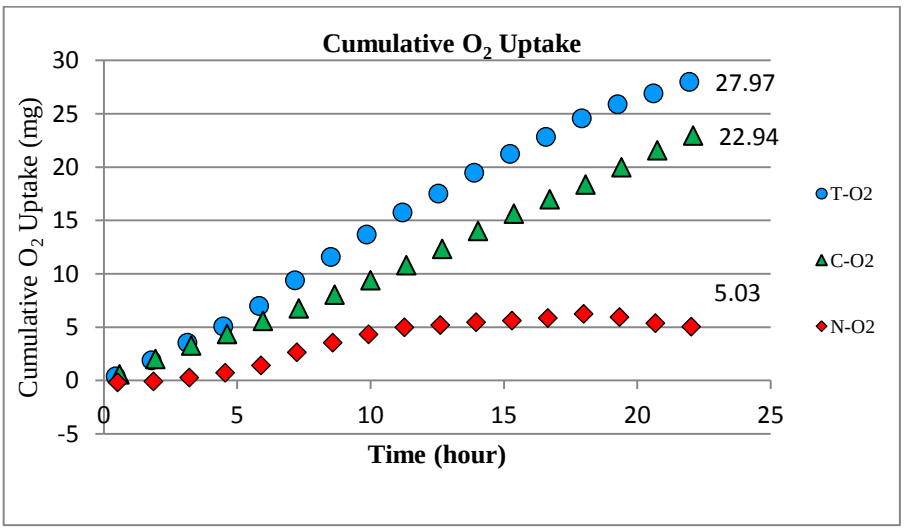


Figure 4.31. T-O_2, C-O_2 and N-O_2 results in Respirometric Test 17 (R2-14.02.2013)

In **Respirometric Test 19**, the Pre-ATU and direct ATU addition were compared. Figure 4.32 shows the cumulative oxygen consumption in test samples. A major difference is seen between the test samples which are previously contacted with ATU and directly contacted with ATU. Addition of ATU to the test samples at the beginning of respirometry tests decreased the cumulative oxygen consumption by suppressing nitrification. Also, analytical results confirmed these respirometric results. On the other hand, the Pre-ATU method gave wrong results since it did not suppress nitrification completely.

Total (T-O_2), carbonaceous (C-O_2) and nitrogenous (N-O_2) oxygen consumption in **Respirometric Test 19** is represented in Figure 4.33. In order to separate N-O_2 and C-O_2, direct ATU samples were used. As seen in Figure 4.33, oxygen consumption due to nitrification is approximately twice higher than oxygen consumption due to organic carbon removal. Nitrification activity in R2 is much higher compared to R1.

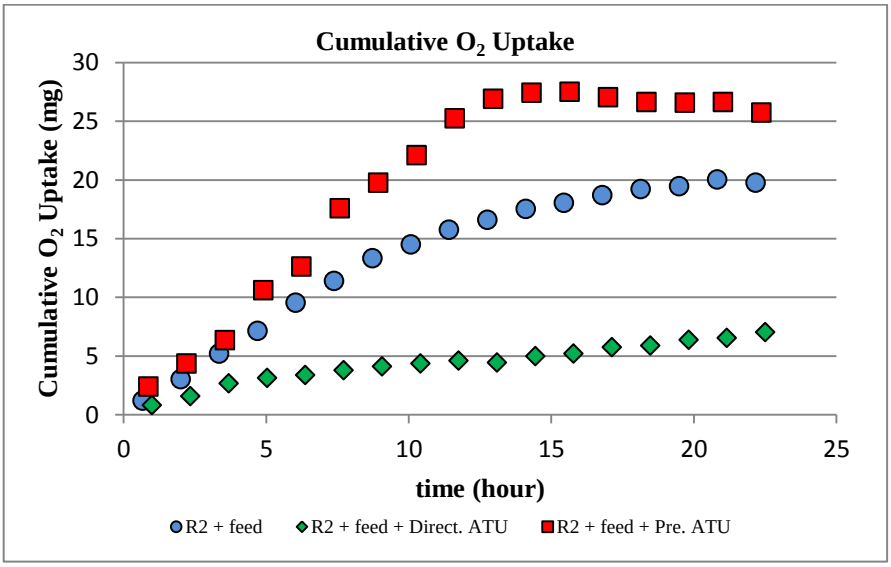


Figure 4.32. Results in Respirometric Test 19 (R2-06.03.2013)

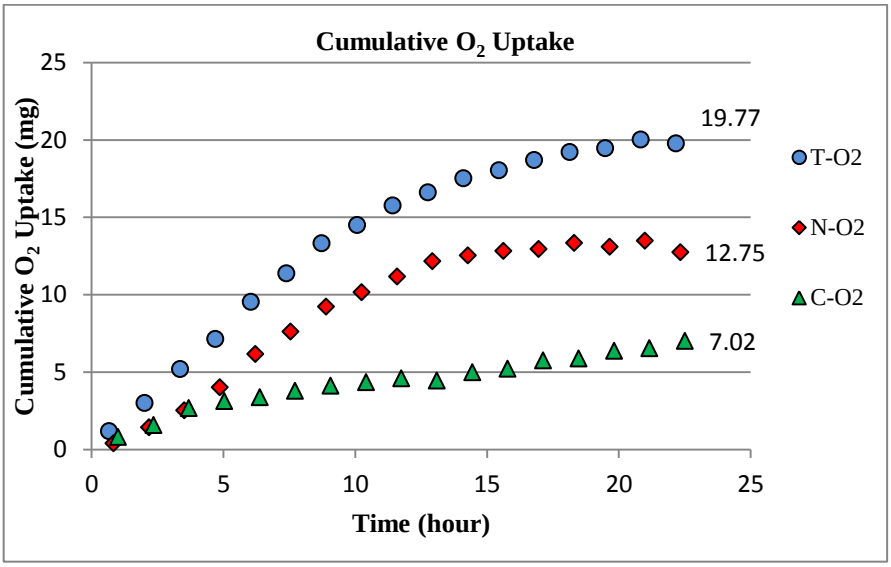


Figure 4.33. T-O_2, N-O_2 and C-O_2 results in Respirometric Test 19 (R2-06.03.2013)

Nitrification contributed to most of the cumulative oxygen consumption in R2 sludge. In **Respirometric Test 26**, nitrification activity in R2 sludge was investigated using ATU and TCMP. Figure 4.34 shows that the nitrification inhibitors, ATU and TCMP, inhibit R2 sludge. In Figure 4.35 N-O_2 values in the presence of both inhibitors are shown. These values are very close to each other. This showed that ATU and TCMP inhibited R2 sludge to the same extent. Lastly, higher concentrations of TCMP did not further inhibit nitrification. Also, Table 4.4 proves that higher TCMP concentrations did not change the average NH_4-N removal. Therefore, 10.6 mg/L TCMP concentration was sufficient for nitrification inhibition.

Table 4.4. Analytical results of Respirometric Test 26

Properties of Test Chambers	Average COD Removal (mg/L)	Average NH_4-N Removal (mg/L)
Control Chamber	188	40
11.6 mg/L ATU	248	13.75
10.6 mg/L TCMP	236	15
21.2 mg/L TCMP	260	13.14
31.8 mg/L TCMP	220	12.88

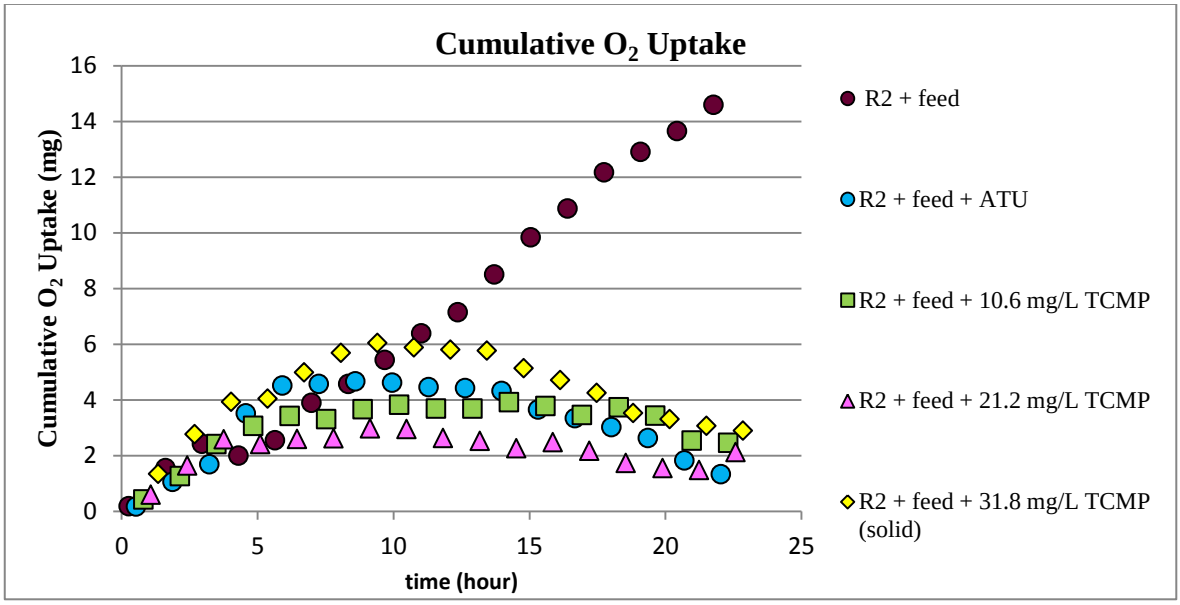


Figure 4.34. Results in Respirometric Test 26 (R2-17.04.2013)

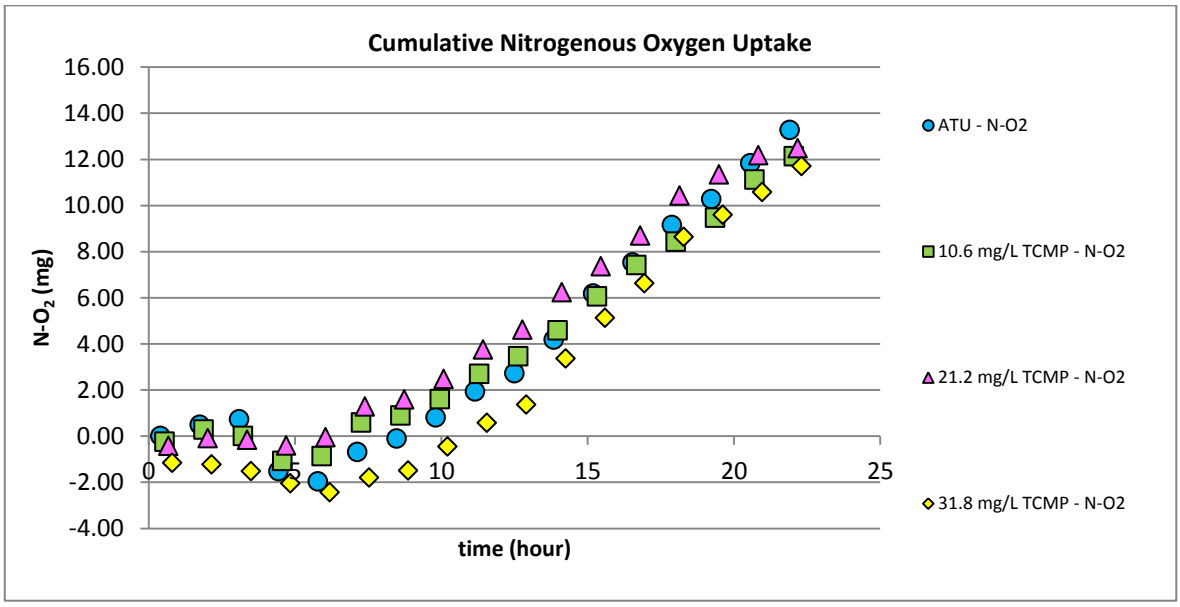


Figure 4.35. $N\text{-}O_2$ results in Respirometric Test 26 (R2-17.04.2013)

4.2.2.3. Results of Reactor 3 (C/N Ratio of 0). As mentioned in previous sections, Reactor 3 was enriched in terms of autotrophic organisms because Feed 3 did not contain any organic substrate. **Respirometric Test 8 and 25** were conducted with this sludge.

When the OUR values of samples which contains a nitrification inhibitor is accepted as 0, T-O_2 equals to N-O_2. As shown in Figure 4.36 and Figure 4.37, OUR values drop to very low levels in some cases such as the presence of nitrification inhibitor. At these times, respirometer records the values as if oxygen transfer occurs from liquid phase to gaseous phase. For this reason, negative values were recorded in oxygen consumption and carbon dioxide production data. In two graphs, R3+ feed represents the T-O_2.

Analytical results of **Respirometric Test 8** support respirometric results. While cumulative O_2 consumption was 10.97 mg O_2 according to respirometric results, using analytical results it was calculated as 10.68 mg O_2.

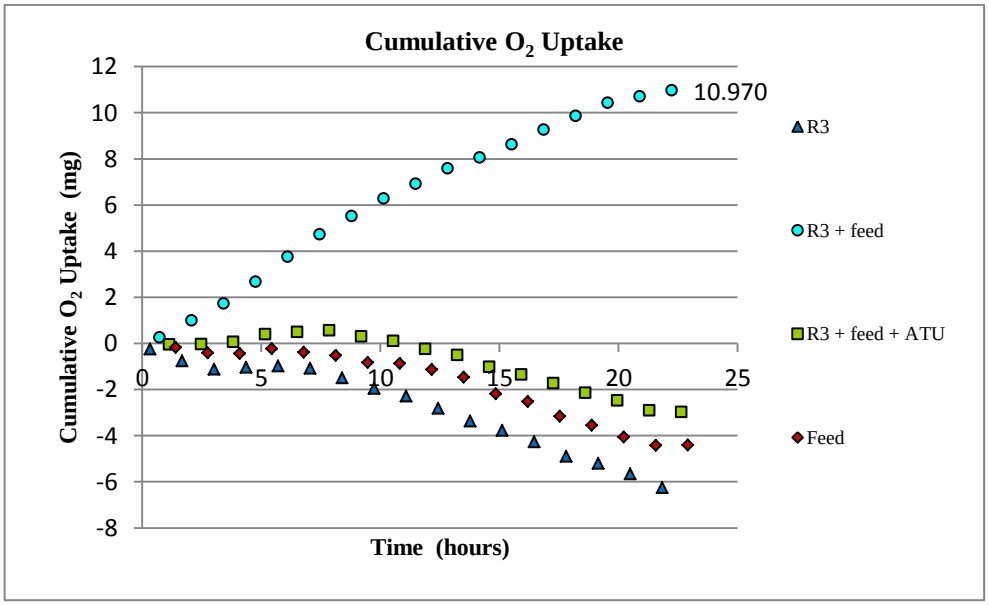


Figure 4.36. Results in Respirometric Test 8 (R3-19.11.2013)

In **Respirometric Test 25**, both ATU and TCMP were used as an inhibitor and both of them affected test samples severely. When analytical measurements were compared with respirometric results, it was shown that theoretical oxygen consumption due to

ammonium removal was 16.34 mg O_2 while the real value measured by respirometry was 14.15 mg O_2.

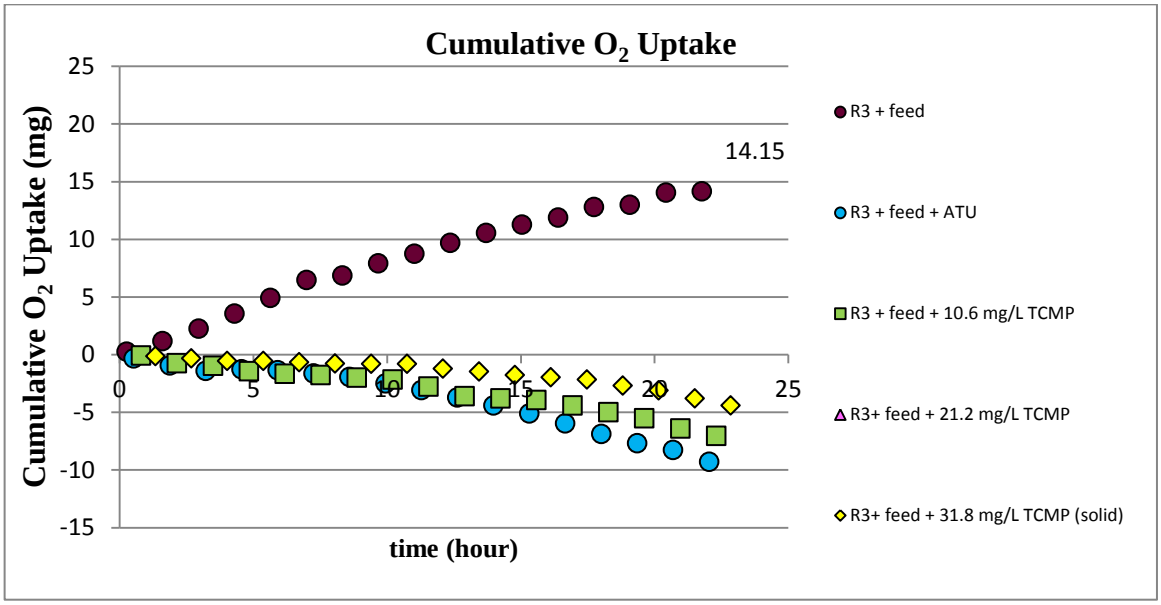


Figure 4.37. Results in Respirometric Test 25 (R3-16.04.2013)

4.2.3. Determination of the Effect of Ag on Activated Sludge

In this part of the study, the aim was to investigate the effect of Ag on different types of activated sludge. Since feeding in these reactors was different from each other, it was expected that the effect of Ag was also significantly different. Furthermore, in the former section, it was observed that a decrease in the C/N ratio provided the enrichment of nitrifiers. If the bacterial population of activated sludge changes significantly with the C/N ratio in a wastewater, it is expected that the inhibitory effect of Ag also changes.

The inhibitory effect of Ag was measured by respirometry tests. Various concentrations of Ag were used for observing the inhibition effect. The properties of these tests are listed in Table 4.1 and Table 4.2.

These respirometry tests mainly measured the total effect of Ag inhibition. In addition to that, in these tests, the aim was to investigate the effect of Ag on heterotrophs and autotrophs, separately. However, when ATU was used for inhibiting nitrifying bacteria, in

the presence of Ag, inhibitory effect of Ag decreased because of complexation with ATU and expected results were not obtained. For this reason, another nitrification inhibitor was selected that did not cause any complexation with Ag. This nitrification inhibitor is TCMP. Also, in this period, the Pre-ATU method was tested. However, this procedure did not give any result.

4.2.3.1. Results of Reactor 1. At the beginning of the study, in order to find the inhibitory range of Ag, high concentrations were applied to R1 and CR. **Respirometric Test 1** was done with the sludge of CR and **Respirometric Test 12** was done with R1 sludge. Figure 4.38 shows the oxygen uptake rates at different Ag concentrations. As shown in Figure 4.38, Ag inhibited sludge samples immediately after the start of test. Concentrations higher than 20 mg/L Ag showed the same inhibitory effect.

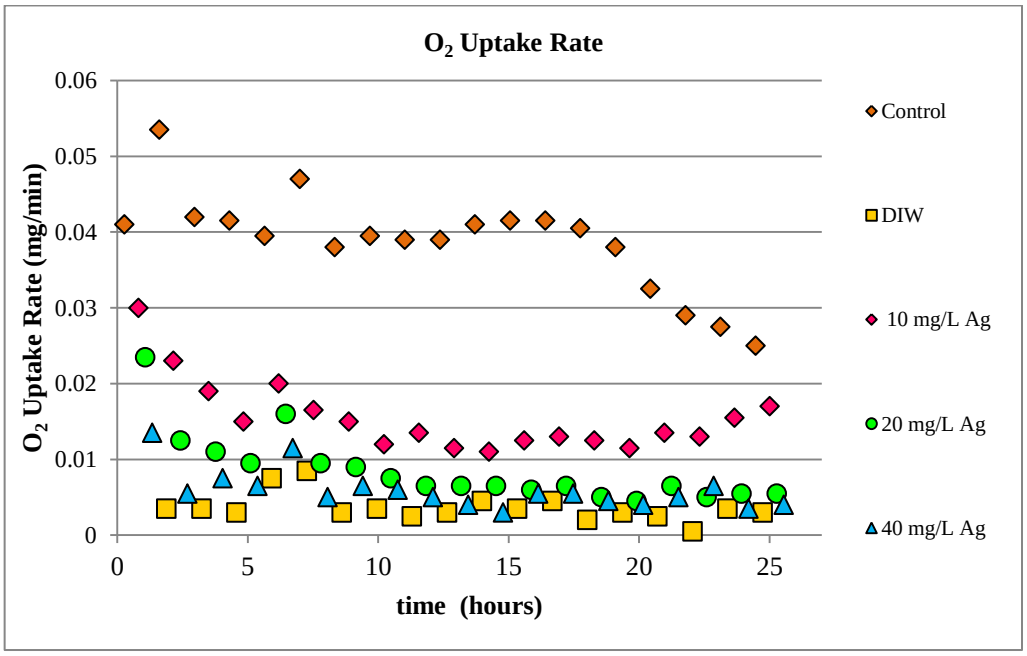


Figure 4.38. Results in Respirometric Test 1 (CR-18.06.2013)

The same test was repeated on 15th January 2013 for comparison. In this experiment at all Ag concentrations (10- 20- 40- 50 mg/L Ag) a severe inhibitory effect was observed. When results were compared with **Respirometric Test 1**, it was observed that the difference aroused from the concentration of biomass in both tests. Table 4.5 shows the MLVSS, cumulative oxygen uptake (T-O_2) and cumulative specific oxygen uptake in

samples. The specific T-O_2 values of control samples were close to each other. But, it seems that in **Respirometric Test 12**, activated sludge samples were completely inhibited by the addition of Ag. Therefore, Ag may be more inhibitory at lower biomass concentrations whereas Table 4.5 shows that there is no linear relation between MLVSS value and inhibitory effect of Ag. For this reason, further investigations are required such as EPS characterization

Table 4.5. Comparison of Respirometric Test 1 and 12

	Test 1	Test 12
Date	18 June 2012	15 January 2013
MLVSS (mg/L)	2172	1210
T-O_2 (mg O_2)	56.6	34.7
Specific T-O_2 (mg O_2/mg MLVSS)	15.7	17.2
Specific T-O_2 at 10 mg/L Ag	6.3	0.9
Specific T-O_2 at 20 mg/L Ag	3.5	0.9
Specific T-O_2 at 40 mg/L Ag	2.5	1

Since **Respirometric Test 1** and **12** showed that high concentrations of Ag severely inhibited R1 and CR sludges. Ag concentrations lower than 5 mg/L were used in **Respirometric Test 14** and **15**. The effect of Ag addition in the range of 0-5 mg/L was investigated in **Respirometric Test 14**. As shown in Figure 4.39, 1 mg/L Ag did not inhibit R1 sludge. Analytical results in **Respirometric Test 14** are shown in Table 4.6. The results show that 2 mg/L Ag affected both organic carbon removal and nitrification. On the other hand, 4 and 5 mg/L Ag completely inhibited R1 sludge. The percentage of inhibition was calculated as follows:

$$\%\ \text{Inhibition} = \frac{\text{OUR}_{\text{control}} - \text{OUR}_{\text{mg/L Ag}}}{\text{OUR}_{\text{control}}} \times 100 \qquad (4.9)$$

Table 4.6. Analytical and respirometric results in Test 14

Ag Concentration (mg/L)	NH_4-N removal (mg/L)	COD removal (mg/L)	Cumulative T-O_2 (mg)	% Inhibition
0	15	486	32.3	-
1	13	468	36.5	0
2	5.13	288	25.4	21.3
4	0.75	0	7	78.3
5	0	0	5.1	84.2

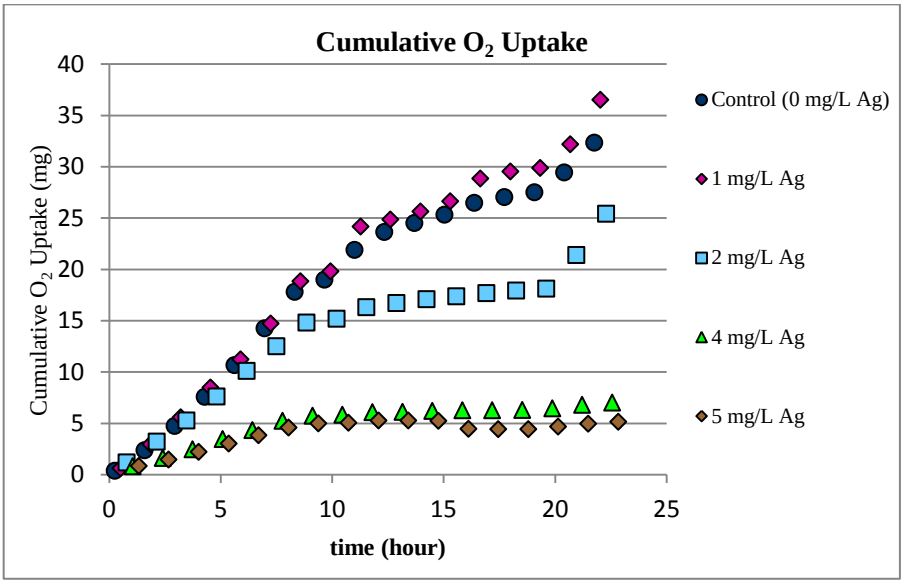


Figure 4.39. Results in Respirometric Test 14 (R1-24.01.2013)

In **Respirometric Test 15**, the same Ag concentrations as in **Respirometric Test 14** were used for inhibiting R1 sludge. However, Figure 4.40 shows that 1 and 2 mg/L Ag had no inhibitory effect on R1 sludge in **Respirometric Test 15**. Also, analytical results in Table 4.7 verifies that test samples containing 2 mg/L Ag had no inhibitory effect. The reason of that can be explained with the condition of sludge taken from the reactor. When activated sludge sample was taken for **Respirometric Test 14**, the biomass in the reactor probably consumed all of the organic substrate in the reactor and it came to endogenous respiration state. Under these conditions, activated sludge was more affected by Ag.

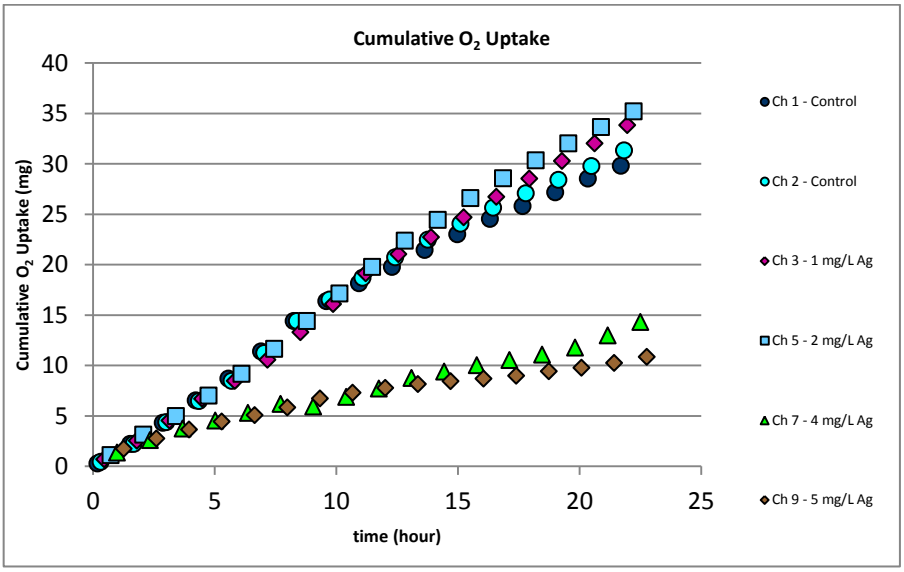


Figure 4.40. Results in Respirometric Test 15 (R1-31.01.2013)

Table 4.7. Analytical and respirometric results of Respirometric Test 15

Ag Concentration (mg/L)	NH_4-N removal (mg/L)	COD removal (mg/L)	T-O_2 (mg)	% Inhibition
0	20.6	359	30.58	-
1	23.25	370	33.84	0
2	19.5	398	35.19	0
4	5.75	33	14.32	53.1
5	5.75	0	10.84	64.5

In respirometry tests, the aim was to observe the effect of Ag on heterotrophs (C-O_2) and autotrophs (N-O_2) separately. For this purpose, nitrification inhibitor ATU was added to same samples which also contained Ag. However, this application did not give good results because ATU and Ag lost their inhibitory effect when they were added at the same time. ATU was used in **Respirometric Test 4**. As shown in Figure 4.43 test chambers containing both ATU and Ag had higher oxygen uptake than test chambers containing only Ag. Thus, wrong readings were obtained in respirometry tests. Also, during test period, colorimetric changes were observed as shown in Figure 4.42. The color of test samples

which contains both ATU and Ag turned to green at the end of respirometry test. Same problems were also observed in **Respirometric Test 3** and **11**.

The short literature review on this issue showed that transition metals like Cu, Ag, Au and Pt form complexes with thiourea derivatives (Ahmad et al., 2002). Also, thiourea derivatives can form complexes with Ag^+ ions at different temperatures. Ag^+ ions bind to sulfur atoms in the structure of thiourea, but binding affinity of Ag^+ ions change with nitrogen atoms in the structure of thiourea (Marco and Zona, 2007). Figure 4.41 shows the Ag –S bonds between thiourea and Ag^+ ions. In Figure 4.41 S represents $C(NH_2)_2$ (Ahmad et al., 2002).

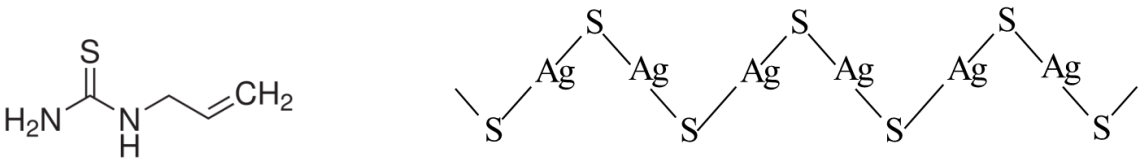


Figure 4.41. Chemical structure of ATU (left) and complex formed with Ag^+ ions (right)

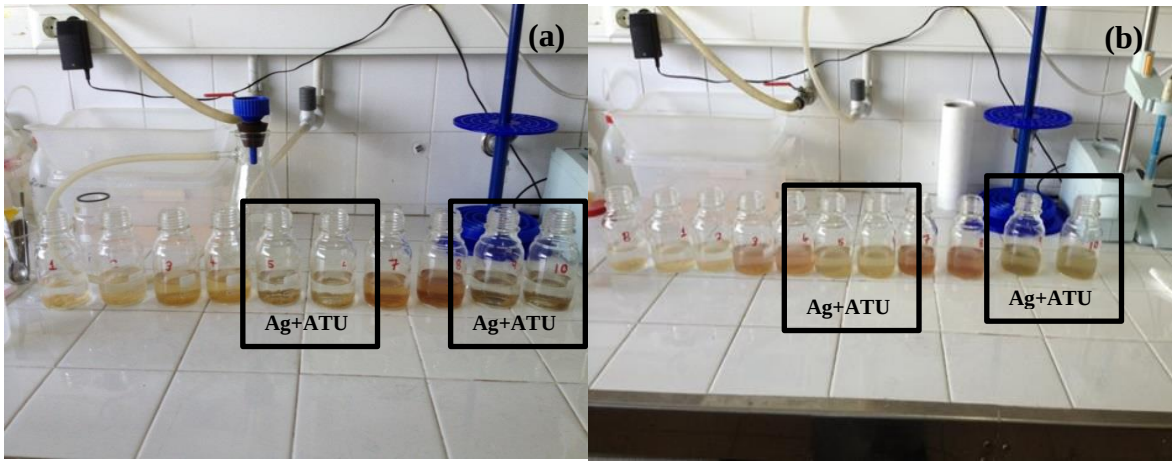


Figure 4.42. Color change in respirometric test chambers (5, 6, 9, 10) (a) at beginning of Test 4 (b) at the end of Test 4

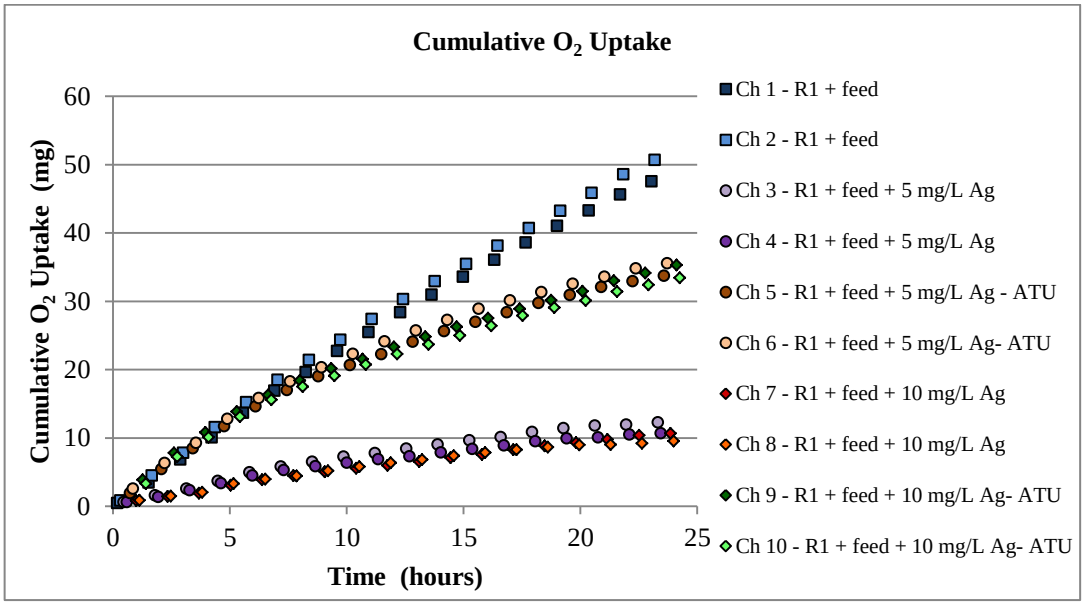


Figure 4.43. Results in Respirometric Test 4 (R1-10.07.2012)

The Pre-ATU method was used in **Respirometric Test 13** and **18** in order to separate the effect of Ag on nitrification and organic carbon removal. In **Respirometric Test 13**, respirometric results showed that 5 mg/L Ag inhibited R1 sludge severely. Also, analytical measurements proved that COD was not removed in Ag- containing samples. The reason is probably disruption of membrane and cell lysis of sludge at 5 mg/L Ag (Choi et al., 2008).

In order to observe the effect of Ag on nitrification and organic carbon removal separately, a lower Ag concentration (2 mg/L Ag) was chosen in **Respirometric Test 18.** This concentration did not affect heterotrophic activity. Although respirometric results showed a slight depletion of oxygen consumption in test chambers which contained Ag and sludge with previously contacted with ATU, analytical results shows that there were no change in nitrogen removal. In this test, Pre-ATU method was used. However, nitrification was not separated with this method. Therefore, $C\text{-}O_2$ and $N\text{-}O_2$ could not be found separately.

In **Respirometric Test 23**, TCMP was used as a nitrification inhibitor for the purpose of separating carbonaceous oxygen consumption and nitrogenous oxygen consumption. Ag concentrations of 2, 3 and 4 mg/L were used in this test. Because R1 sludge has a low autotrophic activity, oxygen consumption due to nitrification could not be recorded by

respirometric measurements, nitrification activity could only be observed through analytical measurements.

Figure 4.44 shows the cumulative oxygen consumption of R1 sludge at different Ag concentrations in **Respirometric Test 23**. 2 mg/L Ag concentration inhibited nitrification, but it caused little inhibition on organic carbon removal. On the other hand, Ag concentrations higher than 2 mg/L suppressed nitrification completely and organic carbon removal was affected very much. Also, it was realized that TCMP had also an inhibitory effect on organic carbon removal in the presence of Ag.

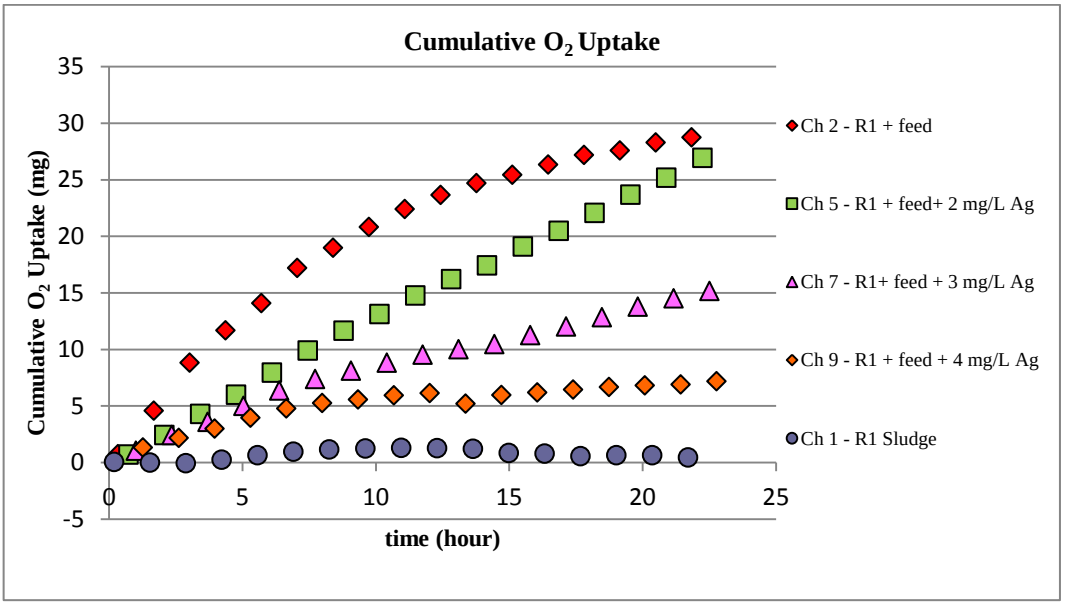


Figure 4.44. T-O_2 results in Respirometric Test 23 (R1-01.04.2013)

4.2.3.2. Results of Reactor 2. **Respirometric Tests 3, 16, 17 and 19** were conducted in order to determine the inhibitory effect of Ag^+ ions on R2 sludge.

In **Respirometric Test 3**, the effect of 5 mg/L Ag was investigated. According to respirometric results 5 mg/L Ag caused 77 % inhibition in R2 Sludge. In Figure 4.45, effect of 5 mg/L Ag on respiration of R2 sludge is shown; control chambers represented the oxygen consumption of sludge under non-inhibitory conditions.

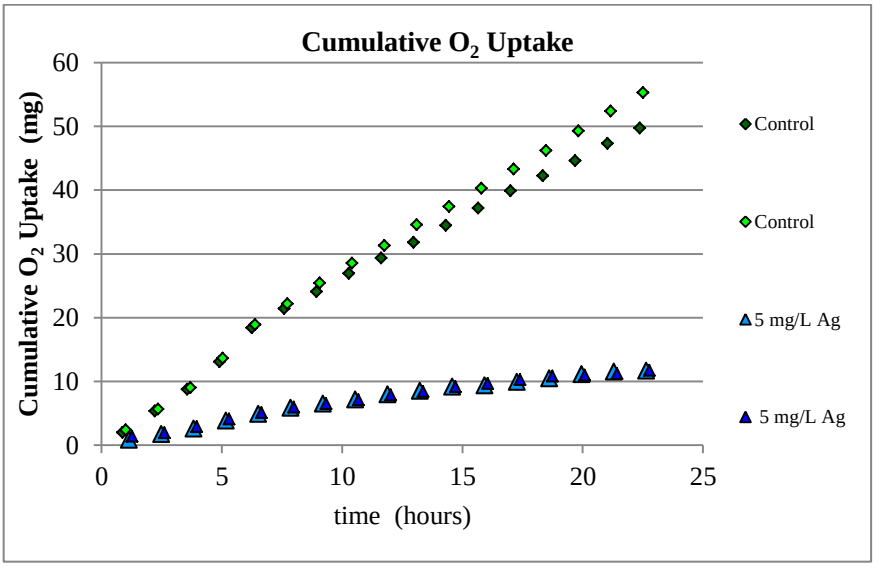


Figure 4.45. T-O_2 Results of Control and 5 mg/L Ag chambers in Respirometric Test 3 (R2-05.07.2012)

Respirometric Test 16 was conducted to find the inhibitory range of Ag for R2 sludge; 0.5, 1, 1.5 and 3 mg/L Ag concentration were used in test chambers. As shown in Figure 4.46, 0.5 mg/L and 1 mg/L Ag decreased cumulative oxygen uptake slightly and 1.5 mg/L Ag affected it more. Lastly, 3 mg/L Ag concentration caused severe inhibition in R2 sludge. However this concentration was less effective in R1 sludge.

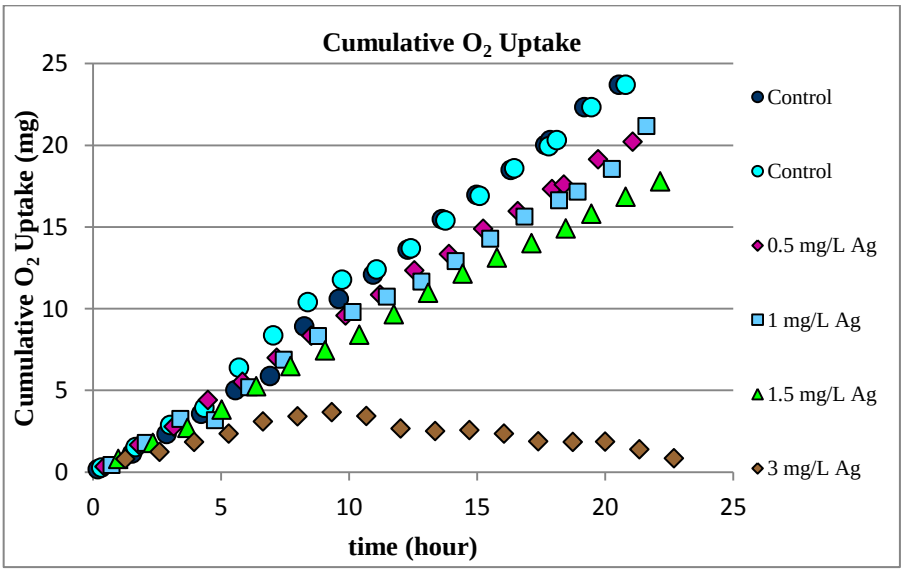


Figure 4.46. T-O_2 results in Respirometric Test 16 (R2-06.02.2013)

Respirometric and analytical results belonging to control chambers in **Respirometric Test 16** were also examined. According to analytical results, 8 mg/L NO_2-N and 37 mg/L NO_3-N were formed as a result of nitrification in control chambers at the end of the respirometry test.With this information theoretical N-O_2 can calculated as follows:

$$\left(8\ \text{mg}\frac{\text{NO}_2-\text{N}}{\text{L}}\times 3.22\ \text{mg O}_2/\text{mg NH}_4-\text{N}\ \times 0.1\ \text{L}\right) + \left(37\text{mg}\frac{\text{NO}_3-\text{N}}{\text{L}}\times 4.33\ \text{mg O}_2/\text{mg NH}_4-\text{N}\ \times 0.1\ \text{L}\right) = 18.6\ \text{mg O}_2\ \text{consumed as a result of nitrification.} \quad (4.10)$$

Respirometric results gave the T-O_2 value as 23.7 mg O_2 for control chambers. Therefore, the carbonaceous oxygen consumption (C-O_2) can be calculated as follows:

$$\text{C}-\text{O}_2 = 23.7\ \text{mg O}_2 - 18.6\ \text{mg O}_2 = 5.1\ \text{mg O}_2\ \text{oxygen consumed due to organic carbon removal.} \quad (4.11)$$

Analytical measurements show that COD removal during the test period was 120 mg COD/L. when Y_h value is assumed as 0.6 (Rittman and McCarty, 2001), the amount of oxygen used for organic carbon removal is theoretically calculated as follows:

$$120\ \text{mg COD/L}\times(1-0.6)\times 0.1\ \text{L sample volume} = 4.8\ \text{mg O}_2\ \text{due to organic carbon removal according to analytical value.} \quad (4.12)$$

This value shows the oxygen consumption due organic carbon removal (C-O_2) and is obtained by making assumptions. It is close to the real respirometric value, 5.1 mg O_2. According to this assumption, 48 mg/L COD was used in energy reactions and 72 mg/L COD was used in cell synthesis. Biomass can be represented in COD unit and 1 mg MLVSS/L is equal to 1.42 mg COD /L approximately (Rittman and McCarty, 2001). According to this value, the following cell synthesis occurs:

$$\frac{72\ \text{mg COD/L}}{\frac{1.42\ \text{mg COD}}{1\ \text{mg MLVSS}}} = 50.7\ \text{mg MLVSS/L} \tag{4.13}$$

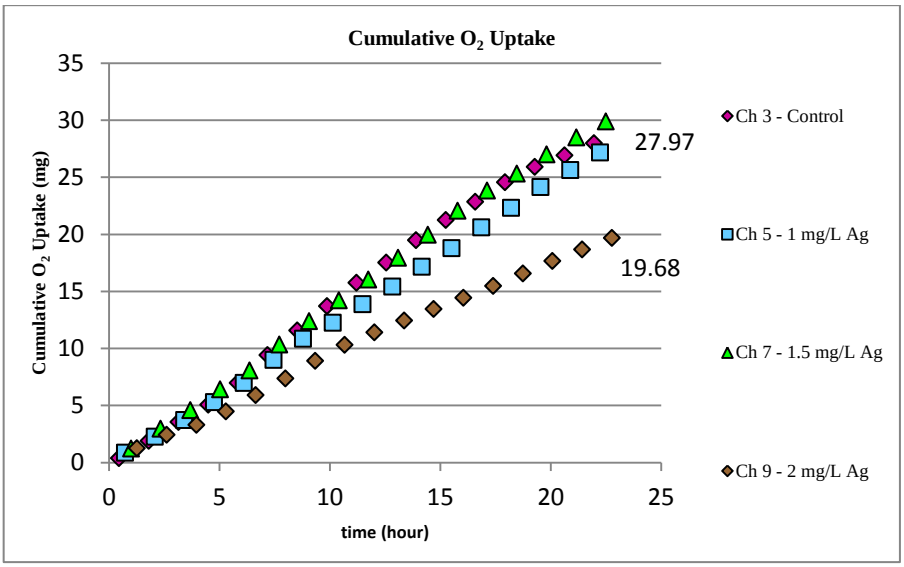


Figure 4.47. T-O_2 results in Respirometric Test 17 (R2-14.02.2013)

In **Respirometric Test 17**, Ag concentrations 1, 1.5 and 2 mg/L were used. Only 2 mg/L Ag affected the respiration of R2 sludge as shown in Figure 4.47. 2 mg/L Ag decreased the cumulative oxygen uptake from 27.9 to 19.7 mg O_2 and inhibited respiration by 29 %. On the other hand, 2 mg/L Ag inhibited completely R2 sludge in **Respirometric Test 19** as shown in Figure 4.48. This result indicates that since activated sludge samples were taken from reactor at different times, the inhibitory effect of Ag changed in accordance to operation of activated sludge.

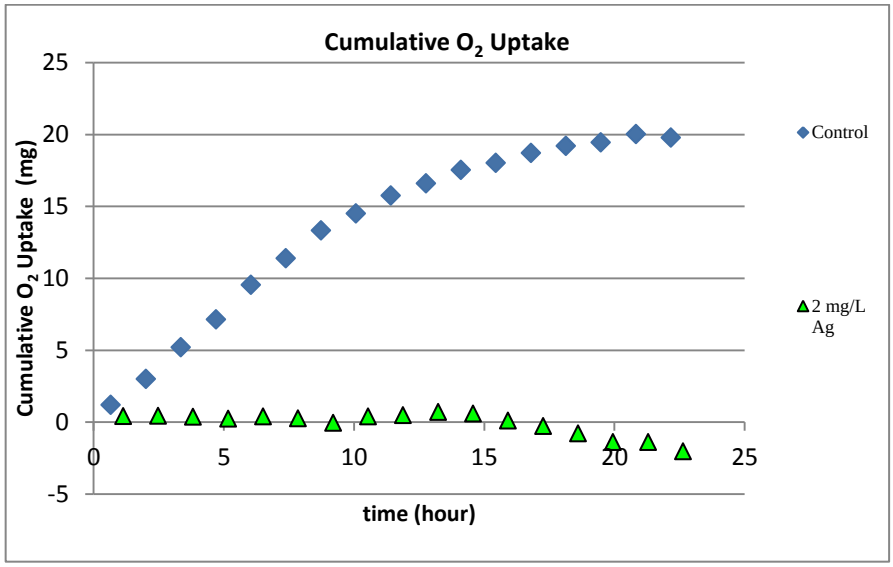


Figure 4.48. Control and 2 mg/L Ag results in Respirometric Test 19 (R2-06.03.2013)

4.2.3.3. Results of Reactor 3. One respirometric test was done with R3 sludge in order to determine effect of Ag on nitrifying sludge. Tests could not be performed further because of the problems in the O_2 sensor. According to previous studies, 0.33 mg/L Ag causes 50 % inhibition (IC_{50}) on the basis of O_2 consumption and 1.01 mg/L Ag concentration causes 90 % inhibition (Çeçen et al., 2010b). With this information, 0.5 and 1 mg/L Ag concentrations were used in the test. ATU was used in this test in order to observe net oxygen consumption due to nitrification. Also, it was observed that ATU did not cause interference at lower Ag concentrations. Since Feed 3 did not contain any organic carbon, measured oxygen uptake aroused from nitrification and endogenous respiration only (T-O_2=N-O_2 +endogenous respiration).

As shown in Figure 4.49, 0.5 mg/L Ag caused 40 % inhibition and 1 mg/L Ag caused 89 % inhibition in R3 sludge. Thus, very low Ag concentrations inhibit R3 sludge severely. Also, Other tests carried out within the scope of a project showed that nitrifying sludge could remove NH_4-N 44 % under the presence of 0.8 mg/L Ag and 0.1 mg/L Ag had nearly no inhibitory effect on R3 sludge while in control chambers 82 % NH_4-N was removed, in 0.1 mg/L Ag added chambers 69 % NH_4-N was removed (Çeçen, 2013)

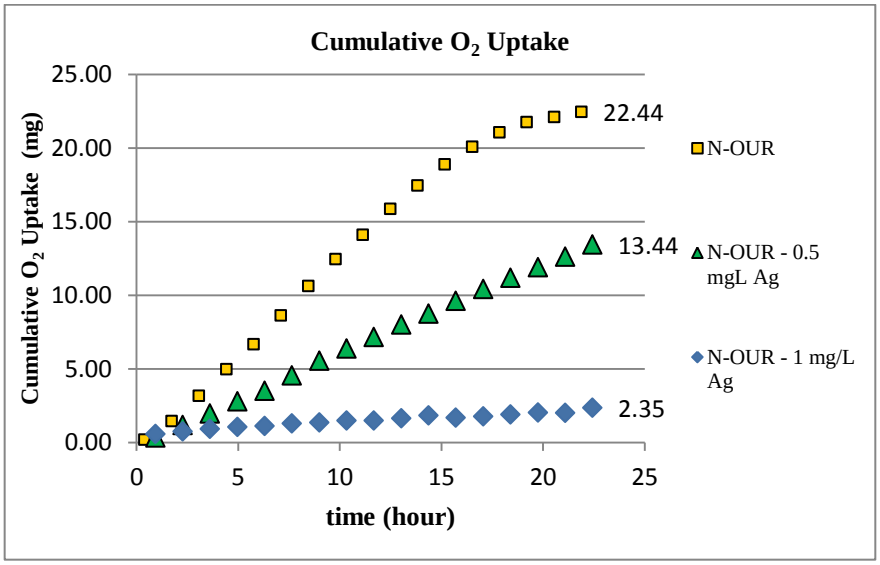


Figure 4.49. Results in Respirometric Test 2 (R3-02.07.2012)

5. CONCLUSIONS AND RECOMMENDATIONS

The objective of this study is to investigate the effect of the C/N ratio in a wastewater on organic carbon removal and nitrification and to study the relationship between the C/N ratio and the inhibitory effect of silver. For these purposes, synthetic wastewaters were prepared and lab-scale activated sludge reactors were fed with these wastewaters. The synthetic wastewaters had C/N ratios of 10, 5 and 0.

After that, respirometry tests were done with activated sludge samples taken from these reactors. In respirometry tests, the effect of C/N ratio on different sludges and the effect of Ag on these sludges were determined. Moreover, in this study, the inhibitory effect of Ag on activated sludge was investigated with regard to nitrification inhibition and inhibition of organic carbon removal.

In order to observe the effect of the C/N ratio on activated sludge reactors, carbonaceous oxygen consumption and nitrogenous oxygen consumption of activated sludge were separated by the aid of nitrification inhibitors. Two types of nitrification inhibitors, ATU and TCMP, were used during the study. Use of ATU in respirometry tests is standardized by different authorities. However, there is no test standard procedure for using TCMP in respirometry tests. These nitrification inhibitors were compared in respirometry tests. Both of them inhibited nitrification activity in activated sludge. TCMP is more advantageous in respirometry tests with Ag since it does not cause any interference with the silver ion.

Respirometry tests demonstrated that the C/N ratio in a wastewater affects the activity of heterotrophs and autotrophs in an activated sludge. In respirometry tests, oxygen consumptions and carbon dioxide productions of heterotrophs and autotrophs were determined. The results show that heterotrophic activity in R1 sludge was highest compared with R2 and R3 sludge because it was fed at a C/N ratio of 10. Also, autotrophic activity was at a minimal level in R1 sludge. On the other hand, since R2 sludge was fed at a C/N ratio of 5, nitrifying activity in R2 sludge was higher than R1 sludge. In R2 sludge, almost half of respiration activity resulted from nitrification. Lastly, because R3 sludge

was fed with a wastewater which did not contain any organic substance, no heterotrophic activity was present and nitrifying bacteria were enriched. Thus, the C/N ratio in wastewater affected nitrogenous oxygen consumption ($N\text{-}O_2$) in the system. With the increase in the C/N ratio of wastewater, depletion was seen in nitrogenous oxygen consumption. The reason is that the population of nitrifying bacteria decreased due to an increase in the C/N ratio. On the other hand, a decrease in the C/N ratio provided more nitrogenous substrates for the enhancement of nitrifiers. Therefore, nitrifying bacteria had chance to remain and grow in the system. Thus, they could compete with heterotrophs for space and oxygen in the system.

In the second part of the study, the effect of Ag on these activated sludges was examined. The results showed that R3 sludge was the most sensitive sludge. 1 mg/L Ag caused complete respiration inhibition in R3 sludge. However, 1 mg/L Ag did not affect R2 sludge and it was observed that R2 sludge could tolerate Ag up to 3 mg/L. Lastly, respirometry tests done with R1 sludge showed that it could tolerate Ag up to 4 - 5 mg/L. 3 mg/L Ag concentration severely affected R2 sludge whereas R1 sludge was slightly affected at this concentration. Complete respiration inhibition in R1 sludge occurred at 5 mg/L Ag concentration.

The inhibitory effect of Ag changed with respect to the feeding of activated sludge. The C/N ratio in wastewater is known to be important for the population of mixed culture in activated sludge. The C/N ratio determines which type of microorganisms grows in the system. Thus, microorganisms that are tolerated high Ag concentrations or more susceptible microorganisms can grow in an activated sludge with respect to C/N ratio.

The respirometric and analytical measurements are not sufficient for assessing the relationship between silver inhibition and the C/N ratio of sludge. Composition of microbial products and metal speciation play also significant role. Therefore, for a detailed analysis, respirometric data should be supported with EPS characterization and metal speciation. Also, it is recommended to study the inhibitory effect of Ag in the presence of single substrates such as glucose and peptone alone.

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APPENDIX A: MONITORING OF ACTIVATED SLUDGE REACTORS

Table A. 1. Operational results of Reactor 1 –July 2012

Run	**1**		**2**		**3**	
Date	**04.07.2012**	**06.07.2012**	**06.07.2012**	**09.07.2012**	**09.07.2012**	**13.07.2012**
pH	8.6	8.91	8.63	9.07	8.82	9.06
Temperature (°C)	25	26.3	25	26.9	27.2	28.9
COD (mg/L)	954	121	1024	168.5	1090	202.5
COD removal (%)	87.3		83.5		81.4	
NH_4-N (mg/L)	50	0.75	37.5	0.25	45	2.5
NH_4-N removal (%)	98.5		99.3		94.4	
MLSS (mg/L)	3630		3630		3420	
MLVSS (mg/L)	2905		2905		2830	
Run period (day)	1.75		2.75		3.75	
F:M Ratio (mg COD/mg MLVSS*day)	0.20		0.13		0.09	
S_0/X_0 (mg COD/mg MLVSS)	0.33		0.35		0.39	
Run	**4**		**5**		**6**	
Date	**13.07.2012**	**23.07.2012**	**23.07.2012**	**25.07.2012**	**25.07.2012**	**01.08.2012**
pH	8.74	8.2	7.89	8.65	8.42	8.94
Temperature (°C)	28.2	26.6	25.4	27.6	26.6	27
COD (mg/L)	1288	9	904	74	470	206
COD removal (%)	99.3		91.8		56.2	
NH4-N (mg/L)	30	0.25	32.5	0.2	30	0.25
NH4-N removal (%)	99.2		99.4		99.2	
MLSS (mg/L)	3420		2310		2212	
MLVSS (mg/L)	2830		1787.5		1703	
Run period (day)	9.79		1.80		6.78	
F:M Ratio (mg COD/mg MLVSS*day)	0.04		0.31		0.09	
S_0/X_0 (mg COD/mg MLVSS)	0.46		0.51		0.28	

Table A. 2. Operational results of Reactor 1 – August 2012

Run	**1**		**2**		**3**	
Date	**01.08.2012**	**03.08.2012**	**03.08.2012**	**06.08.2012**	**06.08.2012**	**08.08.2012**
pH	7.85	8.26	8.56	8.56	8.51	8.84
Temperature (°C)	25.8	27.8	27.3	28.5	27	28.7
COD (mg/L)	934	93	680	76	660	55
COD removal (%)	90.0		88.8		91.7	
NH_4-N (mg/L)	152.5	0.5	152.5	0.5	52.5	0
NH_4-N removal (%)	99.7		99.7		100.0	
Alkalinity (mg as $CaCO_3$/L)						
MLSS (mg/L)	2212		2212		2222	
MLVSS (mg/L)	1703		1703		1948	
Run period (day)	1.79		2.96		2.16	
F:M Ratio (mg COD/mg MLVSS*day)	0.33		0.20		0.24	
S_0/X_0 (mg COD/mg MLVSS)	0.55		0.40		0.34	
Run	**4**		**5**		**6**	
Date	**08.08.2012**	**10.08.2012**	**10.08.2012**	**13.08.2012**	**13.08.2012**	**15.08.2012**
pH	8.51	8.84	8.6	9	8.6	8.94
Temperature (°C)	27	28.7	26.6	25.9	25.3	25.9
COD (mg/L)	883	89	792	102	755	94
COD removal (%)	89.9		87.1		87.6	
NH_4-N (mg/L)	55	0.5	42.5	0.75	25	0.25
NH_4-N removal (%)	99.1		98.2		99.0	
Alkalinity (mg as $CaCO_3$/L)			2362.5	3307.5	2800	2500
MLSS (mg/L)	2222		2222		2306	
MLVSS (mg/L)	1948		1948		1956	
Run period (day)	1.93		2.85		1.81	
F:M Ratio (mg COD/mg MLVSS*day)	0.27		0.18		0.28	
S_0/X_0 (mg COD/mg MLVSS)	0.45		0.41		0.39	

Table A. 3. Operational results of Reactor 1 – September 2012

Run	**1**		**2**		**3**	
Date	**05.09.2012**	**07.09.2012**	**07.09.2012**	**10.09.2012**	**10.09.2012**	**12.09.2012**
pH	8.58	8.69	8.32	8.67	8.51	8.63
Temperature (°C)	24	24.6	24	23.7	23.4	23.4
COD (mg/L)	623.5	235	859	285	1026	360
COD removal (%)	62.3		66.8		64.9	
NH_4-N (mg/L)	52.5	1.5	60.75	1.75	57.5	2.5
NH_4-N removal (%)	97.1		97.1		95.7	
MLSS (mg/L)	3194		3194		2922	
MLVSS (mg/L)	2668		2668		2454	
Run period (day)	1.96		2.97		1.84	
F:M Ratio (mg COD/mg MLVSS*day)	0.19		0.13		0.22	
S_0/X_0 (mg COD/mg MLVSS)	0.23		0.32		0.42	
Run	**4**		**5**		**6**	
Date	**12.09.2012**	**17.09.2012**	**17.09.2012**	**19.09.2012**	**19.09.2012**	**21.09.2012**
pH	8.4	8.72	8.42	8.77	8.31	8.59
Temperature (°C)	23.5	25	24.4	24.3	23.6	23.5
COD (mg/L)	941	495	1078	508	1056	558
COD removal (%)	47.4		52.9		47.2	
NH_4-N (mg/L)	60	4	67.5	4	63.7	5.25
NH_4-N removal (%)	93.3		94.1		91.8	
MLSS (mg/L)	2922		2922		2922	
MLVSS (mg/L)	2454		2454		2454	
Run period (day)	4.95		1.96		1.85	
F:M Ratio (mg COD/mg MLVSS*day)	0.08		0.21		0.22	
S_0/X_0 (mg COD/mg MLVSS)	0.38		0.44		0.43	

Table A. 4. Operational results of Reactor 1 –January 2013

Run	**1**		**2**	
Date	**14.01.2013**	**21.01.2013**	**21.01.2013**	**28.01.2013**
pH	7.9	7.93	7.84	7.92
Temperature(^{o}C)	18.9	23.8	22.5	22.7
COD (mg/L)	660	41	1024	168.5
COD removal (%)	93.8		83.5	
NH_4-N (mg/L)	104	0	100.5	0.5
NH_4-N removal (%)	100		99.5	
MLSS (mg/L)	3535		3075	
MLVSS (mg/L)	2900		2500	
Run period (day)	2.90		2.98	
F/M Ratio (mg COD/mg MLVSS * day)	0.20		0.13	
S_0/X_0 (mg COD/mg MLVSS)	0.23		0.41	

Table A. 5. Operational results of Reactor 1 – February 2013

Run	**1**		**2**		**3**		**4**	
Date	**28.01.2013**	**04.02.2013**	**04.02.2013**	**11.02.2013**	**11.02.2013**	**18.02.2013**	**18.02.2013**	**25.02.2013**
pH	7.71	8.13	7.94	8.06	7.65	8.4	8.64	8.66
Temperature (^{o}C)	22	24.5	23	24.5	19.2	24.1	23.9	23.6
COD (mg/L)	1021	30	1045	50	1065	24	668	33
COD removal (%)	97.1		95.2		97.7		95.1	
NH_4-N (mg/L)	95.5	0	84.5	0.25	102	0	107.5	0.25
NH_4-N removal (%)	100.0		99.7		100.0		99.8	
MLSS (mg/L)	2485		2695		3395		5060	
MLVSS (mg/L)	2020		2220		2680		4040	
Run period (day)	2.97		2.90		2.90		2.96	
F:M Ratio (mg COD/mg MLVSS*day)	0.17		0.16		0.13		0.08	
S_0/X_0 (mg COD/mg MLVSS)	0.51		0.47		0.40		0.17	

Table A. 6. Operational results of Reactor 1 – March 2013

Run	**1**		**2**		**3**		**4**	
Date	**25.02.2013**	**07.03.2013**	**07.03.2013**	**11.03.2013**	**21.03.2013**	**25.03.2013**	**25.03.2013**	**27.03.2013**
pH	7.84	8.82	7.91	8.85	7.96	8.82	8.12	8.76
Temperature (°C)	22.6	24.4	22.6	24.8	22.9	26.2	20	20.7
COD (mg/L)	1007	73	1072	92	1014	108	1099	163
COD removal (%)	92.8		91.4		89.3		85.2	
MLSS (mg/L)	5455		5055		4630		4855	
MLVSS (mg/L)	4485		4025		3695		3880	
Run period (day)	2.70		3.83		3.96		1.92	
F:M Ratio (mg COD/mg MLVSS*day)	0.08		0.06		0.07		0.13	
S0/X0 (mg COD/mg MLVSS)	0.22		0.27		0.27		0.28	

Table A. 7. Operational results of Reactor 1 – April 2013

Run	**1**		**2**		**3**		**4**	
Date	**03.04.2013**	**05.04.2013**	**10.04.2013**	**12.04.2013**	**26.04.2013**	**29.04.2013**	**29.04.2013**	**02.05.2013**
pH	8.78	8.65	8.6	8.25	7.13	8.09	7.42	8.05
Temperature (°C)	23.2	23.9	22.1	20.6	23	22.4	22.9	20.6
COD (mg/L)	257	96	409	63	1087	37	1206	81
COD removal (%)	62.6		84.6		96.6		93.3	
MLSS (mg/L)	5695		4150		3615		3695	
MLVSS (mg/L)	4580		3395		3010		3265	
Run period (day)	1.79		1.83		2.79		2.89	
F:M Ratio (mg COD/mg MLVSS*day)	0.12		0.16		0.12		0.11	
S0/X0 (mg COD/mg MLVSS)	0.06		0.12		0.36		0.37	

Table A. 8. Operational results of Reactor 1 – May 2013

Run	**1**		**2**		**3**		**4**	
Date	**06.05.2013**	**08.05.2013**	**13.05.2013**	**15.05.2013**	**22.05.2013**	**24.05.2013**	**27.05.2013**	**29.05.2013**
pH	7.3	8.22	8.14	8.51	8.3	8.52	8.08	8.31
Temperature (°C)	22.8	20.2	20.8	20.2	23.5	25.1	22.9	22.3
COD (mg/L)	715	33	825	77	1074	140	992	219
COD removal (%)	95.4		90.7		87.0		77.9	
MLSS (mg/L)	3540		3605		3535		2840	
MLVSS (mg/L)	3115		3085		3010		2350	
Run period (day)	1.87		1.98		1.97		1.99	
F:M Ratio (mg COD/mg MLVSS*day)	0.17		0.16		0.17		0.21	
S_0/X_0 (mg COD/mg MLVSS)	0.23		0.27		0.36		0.42	

Table A. 9. Operational results of Reactor 1 – June 2013

Run	**1**		**2**		**3**		**4**	
Date	**03.06.2013**	**05.06.2013**	**10.06.2013**	**12.06.2013**	**17.06.2013**	**19.06.2013**	**24.06.2013**	**26.06.2013**
pH	8.48	8.94	7.27	8.33	8.13	8.74	7.68	8.54
Temperature (°C)	22.9	22.6	23.4	22.7	23.9	24.8	24.4	25.2
COD (mg/L)	1032	86	793	63	948	84	905	96
COD removal (%)	91.7		92.1		91.1		89.4	
MLSS (mg/L)	3420		3525		3950		3830	
MLVSS (mg/L)	2725		2805		3220		3085	
Run period (day)	1.92		1.78		1.89		1.93	
F:M Ratio (mg COD/mg MLVSS*day)	0.19		0.20		0.16		0.17	
S_0/X_0 (mg COD/mg MLVSS)	0.38		0.28		0.29		0.29	

Table A. 10. Operational results of Reactor 2 –July 2012

Run	**1**		**2**		**3**	
Date	**04.07.2012**	**06.07.2012**	**06.07.2012**	**09.07.2012**	**09.07.2012**	**13.07.2012**
pH	8.58	9.01	8.7	9.17	8.87	9.02
Temperature (^{o}C)	24.5	26	24	26.7	25.3	28.5
COD (mg/L)	417	169	866	283	647	327.5
COD removal (%)	59.5		67.3		49.4	
NH_4-N (mg/L)	46.25	1	80	0.25	56	2.5
NH_4-N removal (%)	97.8		99.7		95.5	
MLSS (mg/L)	3480		3480		3110	
MLVSS (mg/L)	2605		2605		2340	
Run period (day)	1.75		2.75		3.75	
F:M Ratio (mg COD/mg MLVSS*day)	0.11		0.07		0.06	
S_0/X_0 (mg COD/mg MLVSS)	0.16		0.33		0.28	
Run	**4**		**5**		**6**	
Date	**13.07.2012**	**23.07.2012**	**23.07.2012**	**25.07.2012**	**25.07.2012**	**01.08.2012**
pH	8.71	8.63	8.09	8.85	8.5	9.12
Temperature (^{o}C)	27.6	26.4	25.2	27.1	26.1	26.7
COD (mg/L)	896	8	432	33.5	194	48
COD removal (%)	99.1		92.2		75.3	
NH_4-N (mg/L)	30	0	32.5		45	0.05
NH_4-N removal (%)	100.0		100.0		99.9	
MLSS (mg/L)	3110		1775		1806	
MLVSS (mg/L)	2340		1307.5		1185	
Run period (day)	9.79		1.80		6.78	
F:M Ratio (mg COD/mg MLVSS*day)	0.02		0.21		0.06	
S_0/X_0 (mg COD/mg MLVSS)	0.38		0.33		0.16	

Table A. 11. Operational results of Reactor 2 –August 2012

Run	**1**		**2**		**3**	
Date	**01.08.2012**	**03.08.2012**	**03.08.2012**	**06.08.2012**	**06.08.2012**	**08.08.2012**
pH	8.12	8.44	8.08	8.43	8.49	8.79
Temperature (^{o}C)	25.7	27.2	26.9	27.8	26.5	28.5
COD (mg/L)	401	27	400	15	336	27
COD removal (%)	93.3		96.3		92.0	
NH_4-N (mg/L)	50	0.25	50	0.25	52.5	0
NH_4-N removal (%)	99.5		99.5		100.0	
Alkalinity (mg as $CaCO_3$/L)						
MLSS (mg/L)	1806		1806		1742	
MLVSS (mg/L)	1185		1185		1386	
Run period (day)	1.79		2.96		2.16	
F:M Ratio (mg COD/mg MLVSS*day)	0.24		0.14		0.17	
S_0/X_0 (mg COD/mg MLVSS)	0.34		0.34		0.24	
Run	**4**		**5**		**6**	
Date	**08.08.2012**	**10.08.2012**	**10.08.2012**	**13.08.2012**	**13.08.2012**	**15.08.2012**
pH	8.2	8.6	8.59	8.83	8.44	8.77
Temperature (^{o}C)	26.6	27.3	26.3	25.7	24.8	25.3
COD (mg/L)	398	42	431	32	394	53
COD removal (%)	89.4		92.6		86.5	
NH_4-N (mg/L)	42.5	0.5	35	0.25	35	0
NH_4-N removal (%)	98.8		99.3		100.0	
Alkalinity (mg as $CaCO_3$/L)			2520	1522.5	1550	1300
MLSS (mg/L)	1742		1742		1802	
MLVSS (mg/L)	1386		1386		1410	
Run period (day)	1.93		2.85		1.81	
F:M Ratio (mg COD/mg MLVSS*day)	0.19		0.13		0.20	
S_0/X_0 (mg COD/mg MLVSS)	0.29		0.31		0.28	

Table A. 12. Operational results of Reactor 2 –September 2012

Run	**1**		**2**		**3**	
Date	**05.09.2012**	**07.09.2012**	**07.09.2012**	**10.09.2012**	**10.09.2012**	**12.09.2012**
pH	7.77	7.39	8	7.13	7.8	6.93
Temperature (°C)	23.3	24.4	23.5	22.9	22.3	22.8
COD (mg/L)	346	64	380	66	518	62
COD removal (%)	81.5		82.6		88.0	
NH_4-N (mg/L)	68	0.25	68	0	75	0.25
NH_4-N removal (%)	99.6		100.0		99.7	
MLSS (mg/L)	2154		2154		1952	
MLVSS (mg/L)	1724		1724		1570	
Run period (day)	1.96		2.97		1.84	
F:M Ratio (mg COD/mg MLVSS*day)	0.30		0.20		0.35	
S_0/X_0 (mg COD/mg MLVSS)	0.20		0.22		0.33	
Run	**4**		**5**		**6**	
Date	**12.09.2012**	**17.09.2012**	**17.09.2012**	**19.09.2012**	**19.09.2012**	**21.09.2012**
pH	7.7	6.11	7.16	5.91	6.7	7.84
Temperature (°C)	22.4	24.7	23.2	23.9	22.3	22.9
COD (mg/L)	382	92	505	104	505	112
COD removal (%)	75.9		79.4		77.8	
NH_4-N (mg/L)	72.5	0.5	75.5	0.5	72.5	0.25
NH_4-N removal (%)	99.3		99.3		99.7	
MLSS (mg/L)	1952		1952		1952	
MLVSS (mg/L)	1570		1570		1570	
Run period (day)	4.95		1.96		1.85	
F:M Ratio (mg COD/mg MLVSS*day)	0.13		0.33		0.35	
S_0/X_0 (mg COD/mg MLVSS)	0.24		0.32		0.32	

Table A. 13. Operational results of Reactor 2 –January 2013

Run	**1**		**2**	
Date	**14.01.2013**	**21.01.2013**	**21.01.2013**	**28.01.2013**
pH	8.48	8.64	8.12	8.88
Temperature (°C)	17.2	26	23.8	27.8
COD (mg/L)	273	4	343	26
COD removal (%)	98.5		92.4	
NH_4-N (mg/L)	102.5	0	83.5	0.25
NH_4-N removal (%)	100		99.7	
MLSS (mg/L)	3685		4175	
MLVSS (mg/L)	2645		2800	
Run period (day)	2.90		2.98	
F:M Ratio (mg COD/mg MLVSS*day)	0.07		0.06	
S_0/X_0 (mg COD/mg MLVSS)	0.10		0.12	

Table A. 14. Operational results of Reactor 2 –February 2013

Run	**1**		**2**		**3**		**4**	
Date	**28.01.2013**	**04.02.2013**	**04.02.2013**	**11.02.2013**	**11.02.2013**	**18.02.2013**	**18.02.2013**	**25.02.2013**
pH	8.36	8.7	8.21	7.95	8.07	5.83	8.09	5.86
Temperature (°C)	23.4	26.6	22.7	26.6	20.5	23.8	26.2	23.3
COD (mg/L)	615	13	471	38	383	8	362	101
COD removal (%)	97.9		91.9		97.9		72.1	
NH_4-N (mg/L)	109	0.25	89	0.25	107.5	2.25	118	20.75
NH_4-N removal (%)	99.8		99.7		97.9		82.4	
MLSS (mg/L)	5090		5705		4480		3140	
MLVSS (mg/L)	3275		3645		2945		2400	
Run period (day)	2.97		2.90		2.90		2.96	
F:M Ratio (mg COD/mg MLVSS*day)	0.05		0.05		0.06		0.07	
S_0/X_0 (mg COD/mg MLVSS)	0.19		0.13		0.13		0.15	

Table A. 15. Operational results of Reactor 2 –March 2013

Run	**1**		**2**		**3**		**4**	
Date	**25.02.2013**	**07.03.2013**	**07.03.2013**	**11.03.2013**	**21.03.2013**	**25.03.2013**	**25.03.2013**	**27.03.2013**
pH	7.25	6.07	7.46	6.14	7.37	5.71	7.67	6.35
Temperature (°C)	22.3	27.3	23.6	23.5	23.1	26.9	20.3	23.9
COD (mg/L)	619	231	826	181	612	48	511	50
COD removal (%)	62.7		78.1		92.2		90.2	
NH_4-N (mg/L)	126.5	4.5	104	14	113	5.25	99	0.5
NH_4-N removal (%)	96.4		86.5		95.4		99.5	
MLSS (mg/L)	3100		3040		1730		1910	
MLVSS (mg/L)	2505		2325		1410		1325	
Run period (day)	2.70		3.84		3.96		1.92	
F:M Ratio (mg COD/mg MLVSS*day)	0.15		0.11		0.18		0.39	
S_0/X_0 (mg COD/mg MLVSS)	0.25		0.36		0.43		0.39	

Table A. 16. Operational results of Reactor 2 –April 2013

Run	**1**		**2**		**3**		**4**	
Date	**03.04.2013**	**05.04.2013**	**10.04.2013**	**12.04.2013**	**26.04.2013**	**29.04.2013**	**29.04.2013**	**02.05.2013**
pH	7.95	7.93	8.46	8.12	7.7	8.39	8.2	8.49
Temperature (°C)	23.7	26.9	23.4	21.8	22	23.7	22	24.3
COD (mg/L)	218	21	116	0	553	34	689	29
COD removal (%)	90.4		100.0		93.9		95.8	
NH_4-N (mg/L)	68.5	0.25	67	0	102	1	102	1
NH_4-N removal (%)	99.6		100.0		99.0		99.0	
MLSS (mg/L)	2960		2800		2945		3250	
MLVSS (mg/L)	2080		1995		2310		2435	
Run period (day)	1.79		1.83		2.79		2.89	
F:M Ratio (mg COD/mg MLVSS*day)	0.27		0.27		0.16		0.14	
S_0/X_0 (mg COD/mg MLVSS)	0.10		0.06		0.24		0.28	

Table A. 17. Operational results of Reactor 2 –May 2013

Run	**1**		**2**		**3**		**4**	
Date	**06.05.2013**	**08.05.2013**	**13.05.2013**	**15.05.2013**	**22.05.2013**	**24.05.2013**	**27.05.2013**	**29.05.2013**
pH	7.57	8.1	8.62	8.35	8.48	8.31	8.17	8.46
Temperature (°C)	22.1	19.9	19.4	20.3	23.5	25.1	22.6	22.5
COD (mg/L)	446	0	372	0	468	10	465	25
COD removal (%)	100.0		100.0		97.9		94.6	
NH_4-N (mg/L)	71	0	55	0.25	57.5	0	60	0
NH_4-N removal (%)	100.0		99.5		100.0		100.0	
MLSS (mg/L)	2730		3240		3785		3705	
MLVSS (mg/L)	2145		2530		2835		2635	
Run period (day)	1.87		1.98		1.97		1.99	
F:M Ratio (mg COD/mg MLVSS*day)	0.25		0.20		0.18		0.19	
S_0/X_0 (mg COD/mg MLVSS)	0.21		0.15		0.17		0.18	

Table A. 18. Operational results of Reactor 2 –June 2013

Run	**1**		**2**		**3**		**4**	
Date	**03.06.2013**	**05.06.2013**	**10.06.2013**	**12.06.2013**	**17.06.2013**	**19.06.2013**	**24.06.2013**	**26.06.2013**
pH	8.11	8.71	7.63	8.21	7.63	8.54	7.47	8.45
Temperature (°C)	24	22.6	22.5	22.6	22.9	24.7	24.1	25.1
COD (mg/L)	451	12	478	18	461	23	440	23
COD removal (%)	97.3		96.2		95.0		94.8	
NH_4-N (mg/L)	61.5	0.25	70	0	66.5	0	63	0
NH_4-N removal (%)	99.6		100.0		100.0		100.0	
MLSS (mg/L)	3285		2905		3155		3365	
MLVSS (mg/L)	2320		2130		2305		2470	
Run period (day)	1.92		1.78		1.90		1.95	
F:M Ratio (mg COD/mg MLVSS*day)	0.22		0.26		0.23		0.21	
S_0/X_0 (mg COD/mg MLVSS)	0.19		0.22		0.20		0.18	

Table A. 19. Operational results of Reactor 3 –July 2012

Run	**1**		**2**		**3**	
Date	**04.07.2012**	**06.07.2012**	**06.07.2012**	**09.07.2012**	**09.07.2012**	**13.07.2012**
pH	7.76	5.71	7.93	6.28	7.75	6.61
Temperature (^{o}C)	25.8	25.9	25.9	26.5	26.4	28.6
NH_4-N (mg/L)	56.3	40.5	282.5	28.5	173	19
NH_4-N removal (%)	28		89.9		89.0	
NH_3-N (FA*) (mg/L)	1.9	0.0	14.3	0.0	6.1	0.1
MLSS (mg/L)	2860		2860		1860	
MLVSS (mg/L)	2175		2175		1450	
Run period (day)	1.89		3.01		3.77	
S_0/X_0 (mg NH_4-N/mg MLVSS)	0.03		0.13		0.12	
Run	**4**		**5**		**6**	
Date	**13.07.2012**	**18.07.2012**	**18.07.2012**	**23.07.2012**	**23.07.2012**	**25.07.2012**
pH	8.26	6.83		7.28	7.95	6.95
Temperature (^{o}C)	28.2	26		26.4	26.4	27.3
NH_4-N (mg/L)	735	0	367	0.5	187.5	1.2
NH_4-N removal (%)	100.0		99.9		99.4	
MLSS (mg/L)	1860		1860		1635	
NH_3-N (FA*) (mg/L)	86.9	0.0	0.0	0.0	10.3	0.0
MLVSS (mg/L)	1450		1450		837.5	
Run period (day)	4.96		4.98		1.82	
S_0/X_0 (mg NH_4-N/mg MLVSS)	0.51		0.25		0.22	

*FA: free ammonia concentration

Table A. 20. Operational results of Reactor 3 –August 2012

Run	**1**		**2**		**3**	
Date	**01.08.2012**	**03.08.2012**	**03.08.2012**	**06.08.2012**	**06.08.2012**	**08.08.2012**
pH	8.4	7.15	8.43	7.54	8.43	7.04
Temperature (°C)	27.4	27.4	26.9	28	26.3	28.4
NH_4-N (mg/L)	300	1	305	20.25	300	2
NH_4-N removal (%)	99.7		93.4		99.3	
NH_3-N (FA*) (mg/L)	44.7	0.0	46.7	0.5	44.3	0.0
Alkalinity (mg as $CaCO_3$/L)						
Alkalinity Consumption (mg as $CaCO_3$/L)						
Alkalinity Consumption (%)						
MLSS (mg/L)	2088		2088		2510	
MLVSS (mg/L)	695		695		982	
Run period (day)	1.79		2.96		2.16	
S_0/X_0 (mg NH_4-N/mg MLVSS)	0.43		0.44		0.31	
Run	**4**		**5**		**6**	
Date	**08.08.2012**	**10.08.2012**	**10.08.2012**	**13.08.2012**	**13.08.2012**	**15.08.2012**
pH	7.62	6.96	8.08	6.97	7.75	6.85
Temperature (°C)	26.1	27.4	25.7	25.7	24.5	25.5
NH_4-N (mg/L)	430	18.75	352.5	13.75	382.5	17.25
NH_4-N removal (%)	95.6		96.1		95.5	
NH_3-N (FA*) (mg/L)	11.1	0.1	24.4	0.1	11.8	0.1
Alkalinity (mg as $CaCO_3$/L)			5092.5	1890	3850.0	1450.0
Alkalinity Consumption (mg as $CaCO_3$/L)					3202.5	2400.0
Alkalinity Consumption (%)					62.9	62.3
MLSS (mg/L)	2510		2510		2294	
MLVSS (mg/L)	982		982		804	
Run period (day)	1.93		2.94		1.85	
S_0/X_0 (mg NH_4-N/mg MLVSS)	0.44		0.36		0.48	

*FA: free ammonia concentration

Table A. 21. Operational results of Reactor 3 –September 2012

Run	**1**		**2**		**3**	
Date	**05.09.2012**	**07.09.2012**	**07.09.2012**	**10.09.2012**	**10.09.2012**	**12.09.2012**
pH	8.05	6.87	7.6	6.78	7.85	6.95
Temperature (^{o}C)	23.1	24.5	22.9	22.9	22	22.9
NH_4-N (mg/L)	487.5	44.25	437.5	44.52	412.5	60
NH_4-N removal (%)	90.9		89.8		85.5	
NH_3-N (FA*) (mg/L)	26.5	0.2	8.6	0.1	13.4	0.3
Alkalinity (mg as $CaCO_3$/L)			5600	1650	4900	1650
Alkalinity Consumption (mg as $CaCO_3$/L)			3950		3250	
Alkalinity Consumption (%)			70.5		66.3	
MLSS (mg/L)	2872		2872		3136	
MLVSS (mg/L)	834		834		838	
Run period (day)	1.96		2.97		1.87	
S_0/X_0 (mg NH_4-N/mg MLVSS)	0.58		0.52		0.49	
Run	**4**		**5**		**6**	
Date	**12.09.2012**	**17.09.2012**	**17.09.2012**	**19.09.2012**	**19.09.2012**	**21.09.2012**
pH	7.4	6.79	7.36	6.76	7.21	6.83
Temperature (^{o}C)	22.5	24.7	22.9	24	21.9	23
NH_4-N (mg/L)	452.5	65	415	55.75	397.5	49.25
NH_4-N removal (%)	85.6		86.6		87.6	
NH_3-N (FA*) (mg/L)	5.5	0.2	4.7	0.2	3.0	0.2
Alkalinity (mg as $CaCO_3$/L)	4900	1300	4500	1600	4150	1200
Alkalinity Consumption (mg as $CaCO_3$/L)	3600		2900		2950	
Alkalinity Consumption (%)	73.5		64.4		71.1	
MLSS (mg/L)	3136		3136		3136	
MLVSS (mg/L)	838		838		838	
Run period (day)	4.95		1.96		1.84	
S_0/X_0 (mg NH_4-N/mg MLVSS)	0.54		0.50		0.47	

*FA: free ammonia concentration

Table A. 22. Operational results of Reactor 3 –January 2013

Run	**1**		**2**		**3**		**4**	
Date	**14.01.2013**	**16.01.2013**	**21.01.2013**	**24.01.2013**	**29.01.2013**	**30.01.2013**	**30.01.2013**	**01.02.2013**
pH	8.19	7.1	8.30	8.03	8.54	8.56	8.45	7.14
Temperature (^{o}C)	18.6	26	21	21.8	17.9	20.7	17.9	26.1
NH_4-N (mg/L)	355	2.5	425	0	287.5	65	277.5	10
NH_4–N removal (%)	99.3		100.0		77.4		96.4	
NH_3-N (FA*) (mg/L)	19.2	0.0	34.3	0.0	31.1	8.8	24.9	0.1
MLSS (mg/L)	7355		5650		3595		3595	
MLVSS (mg/L)	1850		1440		790		790	
Run period (day)	1.99		2.95		0.99		1.95	
S_0/X_0 (mg NH_4-N/mg MLVSS)	0.19		0.30		0.36		0.35	

*FA: free ammonia concentration

Table A. 23. Operational results of Reactor 3 –February 2013

Run	**1**		**2**		**3**		**4**	
Date	**04.02.2013**	**08.02.2013**	**11.02.2013**	**15.02.2013**	**18.02.2013**	**20.02.2013**	**25.02.2013**	**27.02.2013**
pH	8.47	6.34	8.33	6.56	8.39	6.54	8.64	7.40
Temperature (^{o}C)	20.7	29.8	19.9	30.4	30.2	30.8	21.6	29.6
NH_4-N (mg/L)	292.5	95	995.00	57.50	497.5	90	745	2.5
NH_4–N removal (%)	67.5		94.2		81.9		99.7	
NH_3-N (FA*) (mg/L)	33.0	0.2	79.4	0.2	85.5	0.3	124.5	0.0
NO_2-N (mg/L)					100	600		
NO_3-N (mg/L)					170	600		
Total N (mg/L)					667.5	690		
HNO_2-N (FNA) (mg/L)**					0.0	0.3		
Alkalinity (mg $CaCO_3$/L)	1522.5	105.0	3727.5	288.8	1942.5	262.5		
Alkalinity Consumption (mg $CaCO_3$/L)	1417.5		3438.8		1680.0			
Alkalinity Consumption (%)	93.1		92.3		86.5			
Alkalinity Consumption/NH_4–N removal (mg $CaCO_3$/L/mg N)	7.2		3.7		4.1			
MLSS (mg/L)	4710		3760		3760		3705	
MLVSS (mg/L)	1150		1050		1050		1110	
Run period (day)	3.76		4.02		1.98		2.00	
S0/X0 (mg NH_4-N/mg MLVSS)	0.25		0.95		0.47		0.67	

*FA: Free ammonia concentration

** FNA: Free nitrous acid

Table A. 24. Operational results of Reactor 3 –March 2013

Run	**1**		**2**		**3**		**4**	
Date	**11.03.2013**	**13.03.2013**	**20.03.2013**	**22.03.2013**	**25.03.2013**	**27.03.2013**	**27.03.2013**	**28.03.2013**
pH	8.60	7.50	8.71	7.58	8.78	7.55	8.79	7.71
Temperature (^{o}C)	27.7	26.4	19.2	27.6	17.3	23.3	17.7	22.1
NH_4-N (mg/L)	605	2.5	765	22.5	815	1	870	8.25
NH_4–N removal (%)	99.6		97.1		99.9		99.1	
NH_3-N (FA*) (mg/L)	133.5	0.1	126.2	0.6	136.7	0.0	152.4	0.2
NO_2-N (mg/L)			200	1000	100	700		
NO_3-N (mg/L)			340	150	110	440		
Total N (mg/L)			1305.0	1172.5	1025.0	1141.0		
HNO_2-N (FNA) (mg/L)**			0.0	0.1	0.0	0.0		
Alkalinity (mg $CaCO_3$/L)			3832.5	787.5	4016.3	472.5		
Alkalinity Consumption (mg $CaCO_3$/L)			3045.0		3543.8			
Alkalinity Consumption (%)			79.5		88.2			
Alkalinity Consumption/NH_4–N removal (mg $CaCO_3$/L/mg N)			4.1		4.4			
MLSS (mg/L)	5760		5220		5220		6485	
MLVSS (mg/L)	1535		1370		1370		1730	
Run period (day)	1.83		1.93		1.92		0.83	
S0/X0 (mg NH_4-N/mg MLVSS)	0.39		0.56		0.59		0.50	

*FA: Free ammonia concentration

** FNA: Free nitrous acid

Table A. 25. Operational results of Reactor 3 –April 2013

Run	**1**		**2**		**3**		**4**	
Date	**03.04.2013**	**05.04.2013**	**10.04.2013**	**12.04.2013**	**26.04.2013**	**29.04.2013**	**29.04.2013**	**30.04.2013**
pH	8.61	7.08	8.41	7.53	8.50	8.31	8.7	7.60
Temperature (^{o}C)	20.2	27.2	19.9	21.5	22.6	23.0	21.7	22.8
NH_4-N (mg/L)	835	6.5	935	37	505	50	480	24.5
NH_4–N removal (%)	99.2		96.0		90.1		94.9	
NH_3-N (FA*) (mg/L)	120.7	0.1	88.3	0.6	68.3	4.7	90.4	0.5
NO_2-N (mg/L)	100	400	40	800	10	410		
NO_3-N (mg/L)	280	650	53	300	2	150		
Total N (mg/L)	1215.0	1056.5	1028.0	1137.0	517.0	610.0		
HNO_2-N (FNA) (mg/L)**	0.0	0.1	0.0	0.1	0.0	0.0		
Alkalinity (mg $CaCO_3$/L)	3753.8	367.5	3963.8	656.3	1706.3	525.0		
Alkalinity Consumption (mg $CaCO_3$/L)	3386.3		3307.5		1181.3			
Alkalinity Consumption (%)			83.4		69.2			
Alkalinity Consumption/NH_4–N removal (mg $CaCO_3$/L/mg N)	4.1		3.7		2.6			
MLSS (mg/L)	5760		5830		2500		2500	
MLVSS (mg/L)	1515		1415		720		720	
Run period (day)	1.79		1.83		2.79		0.84	
S0/X0 (mg NH_4-N/mg MLVSS)	0.55		0.66		0.70		0.67	

*FA: Free ammonia concentration

** FNA: Free nitrous acid

Table A. 26. Operational results of Reactor 3 –May 2013

Run	**1**		**2**		**3**		**4**	
Date	**06.05.2013**	**07.05.2013**	**13.05.2013**	**14.05.2013**	**22.05.2013**	**24.05.2013**	**27.05.2013**	**28.05.2013**
pH	8.32	8.48	7.76	6.51	8.47	7.09	7.17	6.3
Temperature (^{o}C)	22.9	21.1	22.4	21.3	23.0	25.0	23.2	23.3
NH_4-N (mg/L)	425	109	228	24.25	208	0	245	63.5
NH_4–N removal (%)	74.4		89.4		100.0		74.1	
NH_3-N (FA*) (mg/L)	40.6	12.9	6.2	0.0	27.2	0.0	1.9	0.1
NO_2-N (mg/L)	5.0	300.0	4.0	190.0	2.0	10.0	22.0	180.0
NO_3-N (mg/L)	15.5		2.4		3.8		37.6	
Total N (mg/L)	445.5	409.0	234.4	214.3	213.8	10.0	304.6	243.5
HNO_2-N (FNA) (mg/L)**	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.2
MLSS (mg/L)	2950		3805		2845		2610	
MLVSS (mg/L)	900		1380		1000		935	
Run period (day)	0.83		1.01		1.97		1.10	
S0/X0 (mg NH_4-N/mg MLVSS)	0.47		0.17		0.21		0.25	

Table A. 27. Operational results of Reactor 3 –June 2013

Run	**1**		**2**		**3**		**4**	
Date	**03.06.1905**	**04.06.2013**	**10.06.2013**	**11.06.2013**	**17.06.2013**	**18.06.2013**	**24.06.2013**	**25.06.2013**
pH	7.55	6.34	7.47	6.21	7.6	6.05	7.6	6.05
Temperature (^{o}C)	23.7	23	23.9	23.2	25.3	24.3	25.3	24.3
NH_4-N (mg/L)	255	27	225	27.5	227.5	20.5	232.5	25
NH_4–N removal (%)	89.4		87.8		91.0		89.2	
NH_3-N (FA*) (mg/L)	4.8	0.0	3.5	0.0	5.3	0.0	5.4	0.0
NO_2-N (mg/L)	4.0	220.0	2.0	120.0				
NO_3-N (mg/L)	13.0		9.0					
Total N (mg/L)	272.0	247.0	236.0	147.5				
HNO_2-N (FNA) (mg/L)**	0.0	0.2	0.0	0.2				
MLSS (mg/L)	2010		2010		2265		2145	
MLVSS (mg/L)	850		850		835		890	
Run period (day)	0.90		0.82		0.92		0.92	
S0/X0 (mg NH_4-N/mg MLVSS)	0.30		0.26		0.27		0.26	

*FA: Free ammonia concentration

** FNA: Free nitrous acid

Table A. 28. Operational results of Control Reactor –July 2012

Run	**1**		**2**		**3**	
Date	**04.07.2012**	**06.07.2012**	**06.07.2012**	**09.07.2012**	**09.07.2012**	**13.07.2012**
pH	8.7	9.13	8.76	9.21	8.79	9.18
Temperature (°C)	23.7	23.9	23.4	26.6	26.9	27.2
COD (mg/L)	988	585	1415	506	1528	421
COD removal (%)	40.8		64.2		72.4	
NH_4-N (mg/L)	46.25	1	107.5	0.5	45	2.5
NH_4-N removal (%)	97.83783784		99.5		94.4	
MLSS (mg/L)	4160		4160		3810	
MLVSS (mg/L)	3275		3275		3030	
Run period (day)	1.75		2.75		3.75	
F:M Ratio (mg COD/mg MLVSS*day)	0.17		0.11		0.09	
S_0/X_0 (mg COD/mg MLVSS)	0.30		0.43		0.50	
Run	**4**		**5**		**6**	
Date	**13.07.2012**	**23.07.2012**	**23.07.2012**	**25.07.2012**	**25.07.2012**	**01.08.2012**
pH	8.68	8.74	8.2	8.8	8.37	9.15
Temperature (°C)	26.8	25.9	24.2	26.5	25.9	26.3
COD (mg/L)	1627	76	819	77	494	73
COD removal (%)	95.3		90.6		85.2	
NH_4-N (mg/L)	30	0.25	62.5	0.45	55	1.5
NH_4-N removal (%)	99.2		99.3		97.3	
MLSS (mg/L)	3810		2340		2340	
MLVSS (mg/L)	3030		1842.5		1842.5	
Run period (day)	9.79		1.80		6.78	
F:M Ratio (mg COD/mg MLVSS*day)	0.03		0.30		0.08	
S_0/X_0 (mg COD/mg MLVSS)	0.54		0.44		0.27	

Table A. 29. Operational results of Control Reactor –August 2012

Run	**1**		**2**		**3**	
Date	**01.08.2012**	**03.08.2012**	**03.08.2012**	**06.08.2012**	**06.08.2012**	**08.08.2012**
pH	8.04	8.83	8.57	8.94	8.86	9.11
Temperature (°C)	25.6	26.4	26.5	27.1	25.8	27.2
COD (mg/L)	589	20	500	30	562	17
COD removal (%)	96.6		94.0		97.0	
NH_4-N (mg/L)	47.5	0.25	52.5	0.25	52.5	0
NH_4-N removal (%)	99.5		99.5		100.0	
MLSS (mg/L)	2566		2566		2674	
MLVSS (mg/L)	1949		1949		2202	
Run period (day)	1.79		2.96		2.16	
F:M Ratio (mg COD/mg MLVSS*day)	0.29		0.17		0.21	
S_0/X_0 (mg COD/mg MLVSS)	0.30		0.26		0.26	
Run	**4**		**5**		**6**	
Date	**08.08.2012**	**10.08.2012**	**10.08.2012**	**13.08.2012**	**13.08.2012**	**15.08.2012**
pH	8.37	8.9	8.8	9.07	8.58	9.02
Temperature (°C)	25.1	27	25.9	24.7	24.2	24.6
COD (mg/L)	699	99	700	84	784	89.5
COD removal (%)	85.8		88.0		88.6	
NH_4-N (mg/L)	70	0.25	45	0.5	47.5	0
NH_4-N removal (%)	99.6		98.9		100.0	
MLSS (mg/L)	2674		2674		2864	
MLVSS (mg/L)	2202		2202		2306	
Run period (day)	1.93		2.85		1.81	
F:M Ratio (mg COD/mg MLVSS*day)	0.24		0.16		0.24	
S_0/X_0 (mg COD/mg MLVSS)	0.32		0.32		0.34	

Table A. 30. Operational results of Control Reactor –September 2012

Run	**1**		**2**		**3**	
Date	**05.09.2012**	**07.09.2012**	**07.09.2012**	**10.09.2012**	**10.09.2012**	**12.09.2012**
pH	8.32	8.7	8.63	8.95	8.57	8.81
Temperature (°C)	23.3	24.4	23.9	22.8	22.6	22.4
COD (mg/L)	387	97	563	97	923	91
COD removal (%)	74.9		82.8		90.1	
NH_4-N (mg/L)	56.25	0.25	60.5	0	63.25	0.25
NH_4-N removal (%)	99.6		100.0		99.6	
MLSS (mg/L)	2846		2846		2970	
MLVSS (mg/L)	2318		2318		2394	
Run period (day)	1.96		2.97		1.84	
F:M Ratio (mg COD/mg MLVSS*day)	0.22		0.15		0.23	
S_0/X_0 (mg COD/mg MLVSS)	0.17		0.24		0.39	
Run	**4**		**5**		**6**	
Date	**12.09.2012**	**17.09.2012**	**17.09.2012**	**19.09.2012**	**19.09.2012**	**21.09.2012**
pH	8.55	8.91	8.63	8.74	8.51	8.74
Temperature (°C)	22.7	24.2	23.5	22.9	22.3	22.5
COD (mg/L)	770	157	825	122	796	134
COD removal (%)	79.6		85.2		83.2	
NH_4-N (mg/L)	61.5	0.75	61	0.25	57.25	0.25
NH_4-N removal (%)	98.8		99.6		99.6	
MLSS (mg/L)	2970		2970		2970	
MLVSS (mg/L)	2394		2394		2394	
Run period (day)	4.95		1.96		1.85	
F:M Ratio (mg COD/mg MLVSS*day)	0.08		0.21		0.23	
S_0/X_0 (mg COD/mg MLVSS)	0.32		0.34		0.33	

Table A. 31. Operational results of Control Reactor –January 2013

Run	**1**		**2**	
Date	**14.01.2013**	**21.01.2013**	**21.01.2013**	**28.01.2013**
pH	7.67	8.21	7.87	8.85
Temperature(^{o}C)	18.9	24.3	22.8	24.4
COD (mg/L)	988	36	1081	23
COD removal (%)	96.4		97.9	
NH_4-N (mg/L)	104.5	0.25	108	1.75
NH_4-N removal (%)	99.8		98.4	
MLSS (mg/L)	2580		2860	
MLVSS (mg/L)	2225		2350	
Run period (day)	2.90		2.98	
F/M Ratio (mg COD/mg MLVSS * day)	0.20		0.14	
S_0/X_0 (mg COD/mg MLVSS)	0.44		0.46	

Table A. 32. Operational results of Control Reactor –February 2013

Run	**1**		**2**		**3**		**4**	
Date	**28.01.2013**	**04.02.2013**	**04.02.2013**	**11.02.2013**	**11.02.2013**	**18.02.2013**	**18.02.2013**	**25.02.2013**
pH	7.89	8.81	8.26	8.77	7.42	8.2	8.33	8.54
Temperature (°C)	23.8	26.2	22.9	25	20.7	22.3	25	22.2
COD (mg/L)	918		1089	56	1067	38	1033	117
COD removal (%)	100.0		94.9		96.4		88.7	
NH_4-N (mg/L)	82.5	0.25	94.5	1.25	100	0.25	107	26
NH_4-N removal (%)	99.7		98.7		99.8		75.7	
MLSS (mg/L)	3150		3110		2820		3370	
MLVSS (mg/L)	2495		2415		2160		2725	
Run period (day)	2.97		2.90		2.90		2.96	
F:M Ratio (mg COD/mg MLVSS*day)	0.13		0.14		0.16		0.12	
S_0/X_0 (mg COD/mg MLVSS)	0.37		0.45		0.49		0.38	

Table A. 33. Operational results of Control Reactor –March 2013

Run	**1**		**2**		**3**		**4**	
Date	**25.02.2013**	**07.03.2013**	**07.03.2013**	**11.03.2013**	**21.03.2013**	**25.03.2013**	**25.03.2013**	**27.03.2013**
pH	7.71	8.82	7.91	8.65	8.33	8.5	8.24	8.18
Temperature (°C)	21.7	24.4	22.6	22.7	23.4	24.2	20.2	22.2
COD (mg/L)	1147	291	1099	227	1127	116	1148	64
COD removal (%)	74.6		79.3		89.7		94.4	
MLSS (mg/L)	4215		3710		4070		4450	
MLVSS (mg/L)	3710		2860		3330		3555	
Run period (day)	2.70		3.83		3.96		1.92	
F:M Ratio (mg COD/mg MLVSS*day)	0.10		0.09		0.08		0.15	
S0/X0 (mg COD/mg MLVSS)	0.31		0.38		0.34		0.32	

Table A. 34. Operational results of Control Reactor –April 2013

Run	**1**		**2**		**3**		**4**	
Date	**03.04.2013**	**05.04.2013**	**10.04.2013**	**12.04.2013**	**26.04.2013**	**29.04.2013**	**29.04.2013**	**02.05.2013**
pH	8.43	8.3	8.24	8.16	7.87	8.7	8.29	8.53
Temperature (°C)	23.8	25.1	23.8	21.9	23.4	25.2	24	23.9
COD (mg/L)	500	143	536	10	1174	28	1218	39
COD removal (%)	71.4		98.1		97.6		96.8	
MLSS (mg/L)	4845		4540		3590		3990	
MLVSS (mg/L)	3825		2670		2970		3375	
Run period (day)	1.79		1.83		2.79		2.89	
F:M Ratio (mg COD/mg MLVSS*day)	0.15		0.20		0.12		0.10	
S0/X0 (mg COD/mg MLVSS)	0.13		0.20		0.40		0.36	

Table A. 35. Operational results of Control Reactor –May 2013

Run	**1**		**2**		**3**		**4**	
Date	**06.05.2013**	**08.05.2013**	**13.05.2013**	**15.05.2013**	**22.05.2013**	**24.05.2013**	**27.05.2013**	**29.05.2013**
pH	7.33	7.98	8.08	8.01	8.06	8.34	8.1	8.55
Temperature (°C)	22.4	20.4	19.9	20.1	23.1	25.3	23.1	22.6
COD (mg/L)	887	6	946	20	940	56	659	104
COD removal (%)	99.3		97.9		94.0		84.2	
MLSS (mg/L)	3675		3800		4160		2530	
MLVSS (mg/L)	3140		3265		3435		2120	
Run period (day)	1.87		1.98		1.97		1.99	
F:M Ratio (mg COD/mg MLVSS*day)	0.17		0.15		0.15		0.24	
S0/X0 (mg COD/mg MLVSS)	0.28		0.29		0.27		0.31	

Table A. 36. Operational results of Control Reactor –June 2013

Run	**1**		**2**		**3**		**4**	
Date	**03.06.2013**	**05.06.2013**	**10.06.2013**	**12.06.2013**	**17.06.2013**	**19.06.2013**	**24.06.2013**	**26.06.2013**
pH	8.34	8.56	7.51	8.32	8.15	8.55	7.51	8.4
Temperature (^{o}C)	22.9	22.7	23.3	23.3	24.4	24.9	24.4	25.6
COD (mg/L)	1048	50	815	66	924	74	873	80
COD removal (%)	95.2		91.9		92.0		90.8	
MLSS (mg/L)	3625		3710		4500		4025	
MLVSS (mg/L)	2930		2880		3650		3270	
Run period (day)	1.92		1.78		1.89		1.93	
F:M Ratio (mg COD/mg MLVSS*day)	0.18		0.20		0.14		0.16	
S0/X0 (mg COD/mg MLVSS)	0.36		0.28		0.25		0.27	

APPENDIX B: RAW DATA IN RESPIROMETRIC TESTS

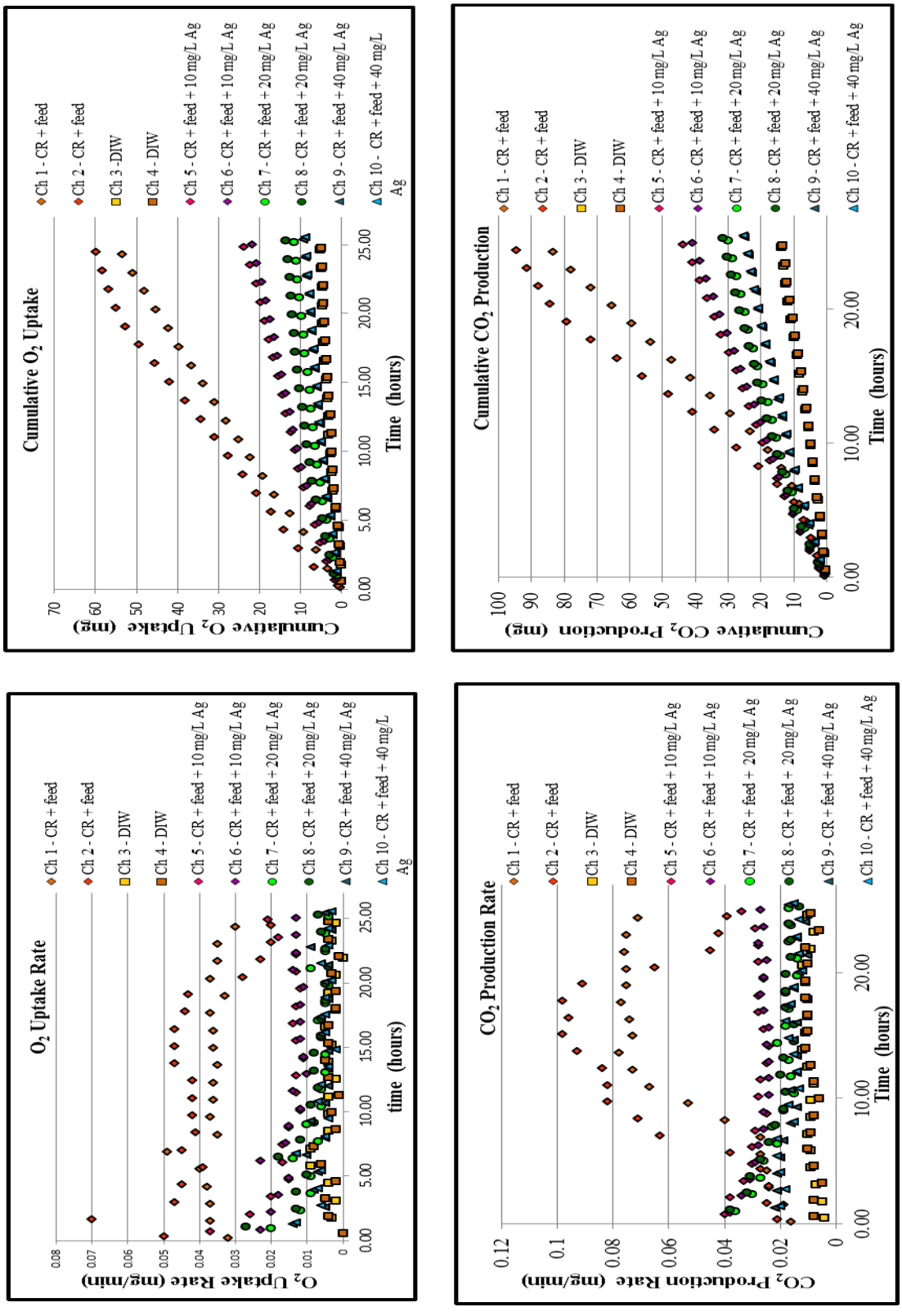


Figure B. 1. Raw data of Test 1 (CR - 18 June 2012) 10-20-40 mg/L Ag

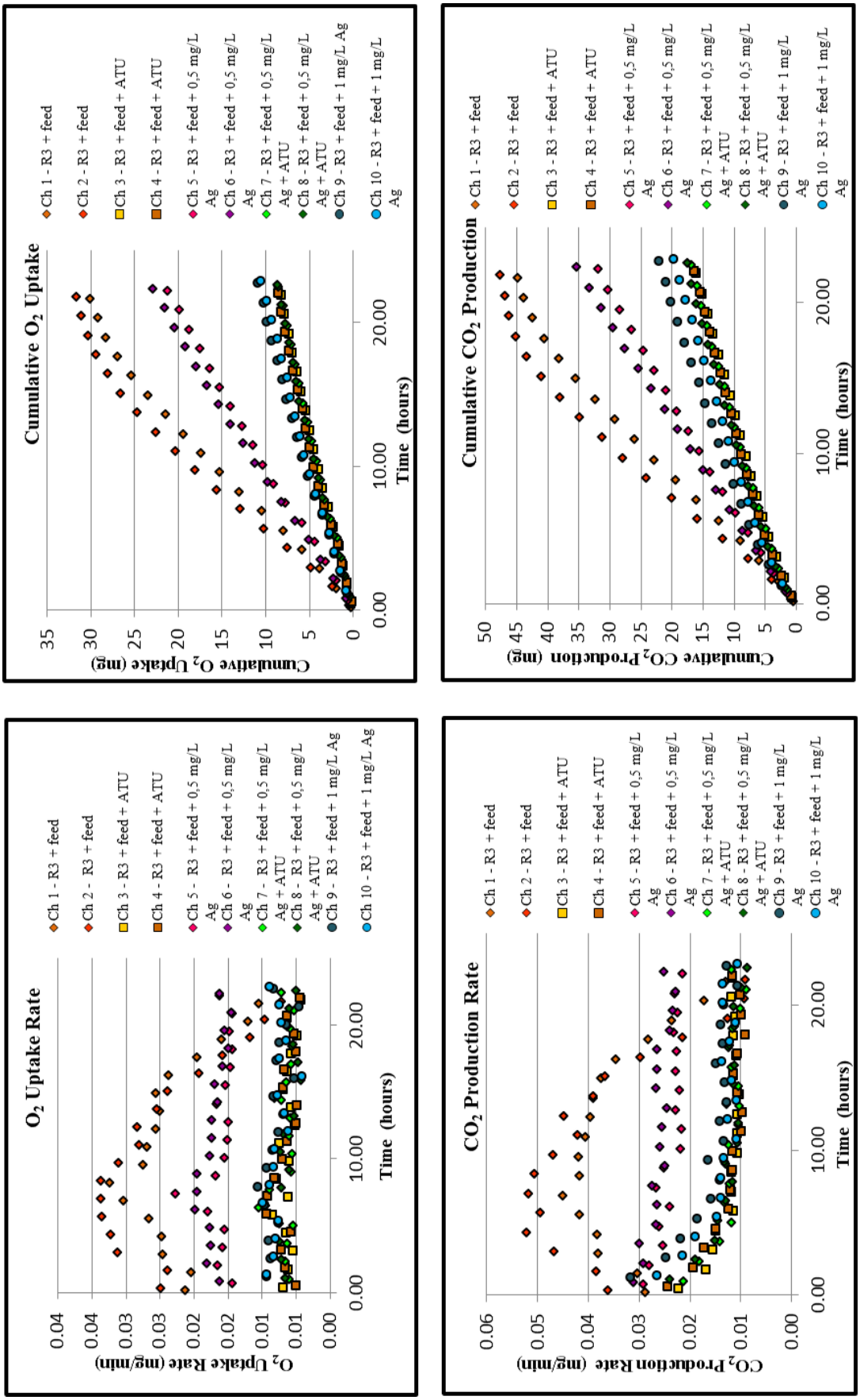


Figure B. 2. Raw data of Test 2 (R3 - 2 July 2012) 0.5-1 mg/L Ag

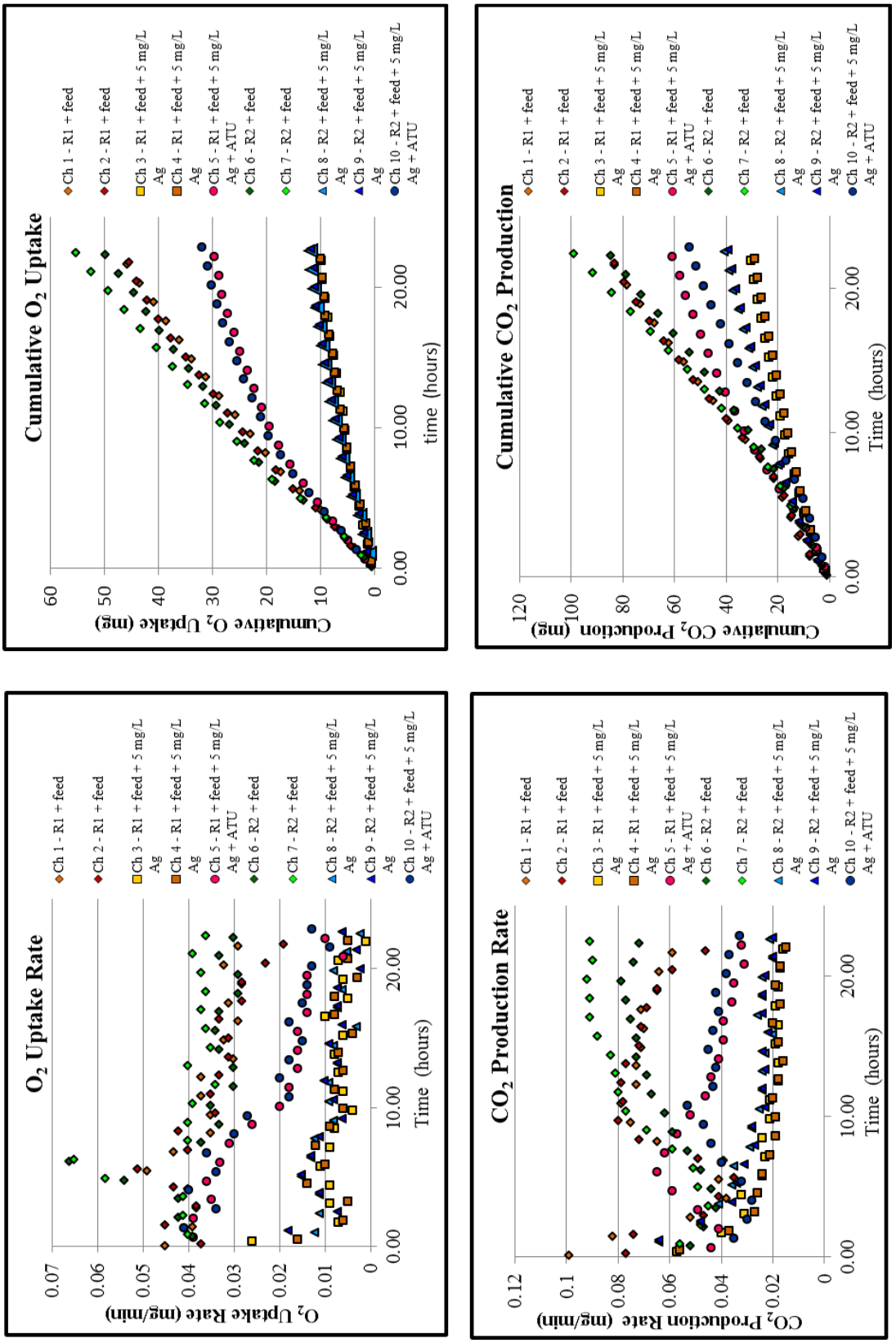


Figure B. 3. Raw data of Test 3 (R1 & R2 - 5 July 2012) 5 mg/L Ag

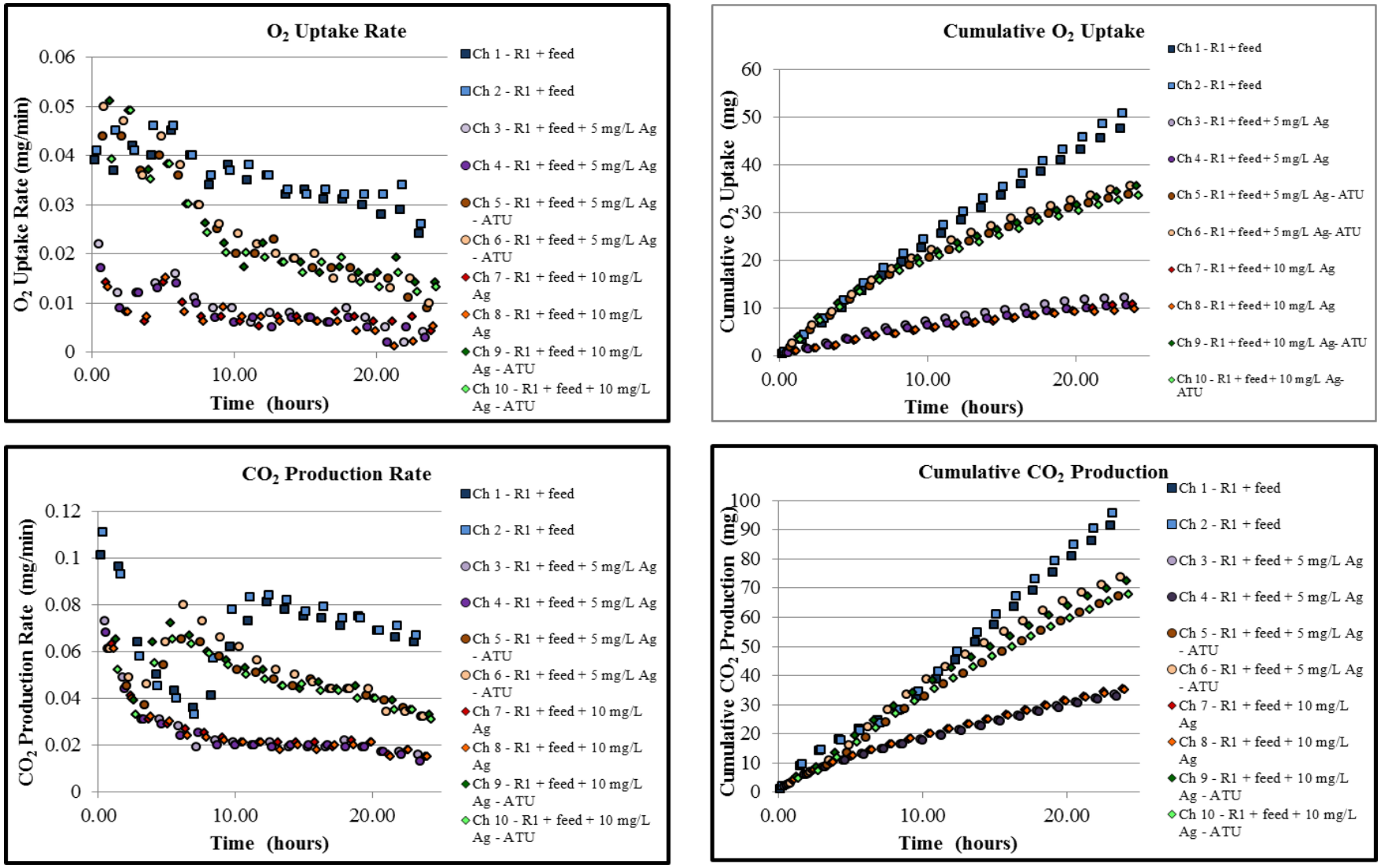


Figure B. 4. Raw data of Test 4 (R1 - 10 July 2012) 5-10 mg/L Ag

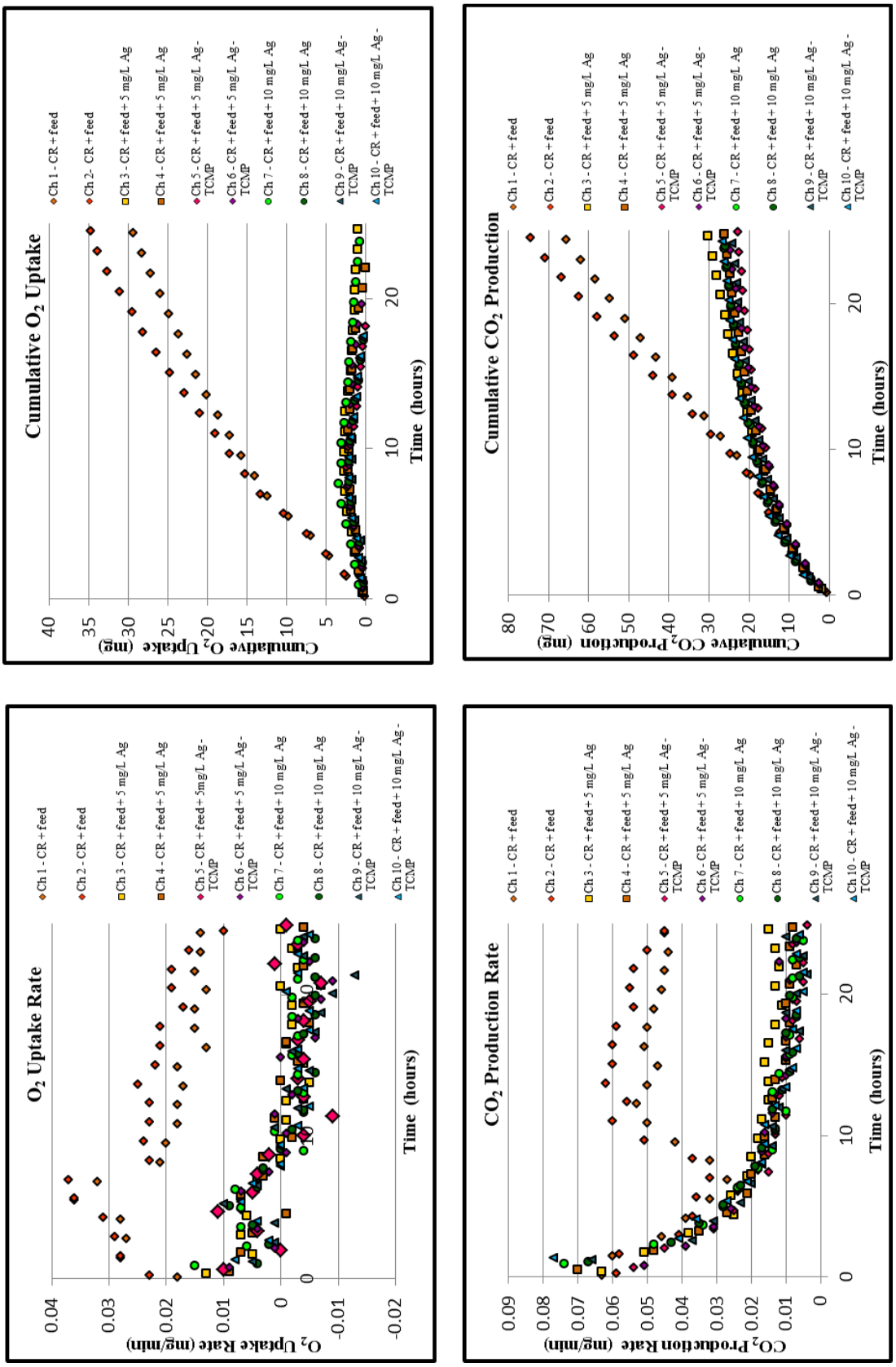
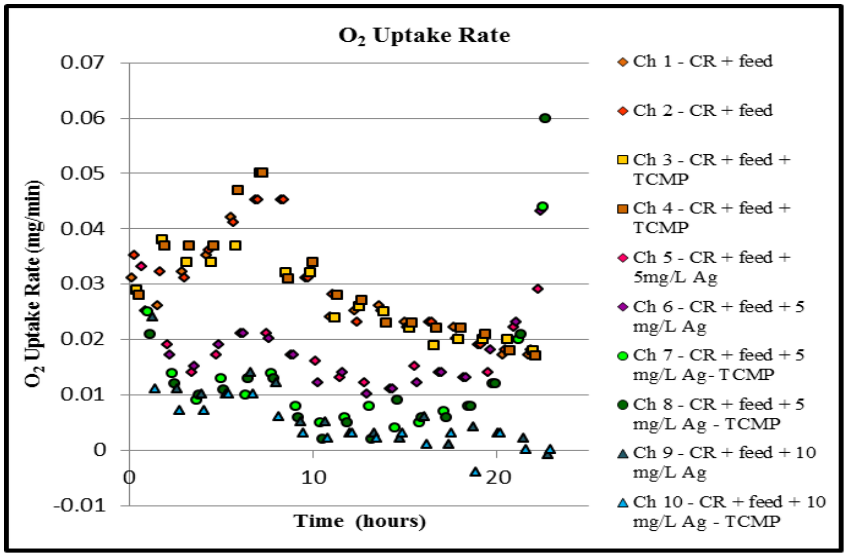
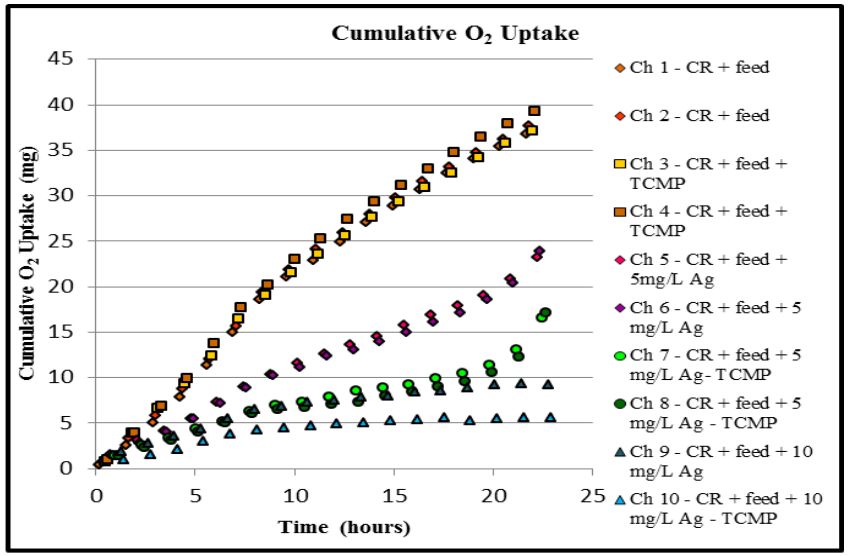
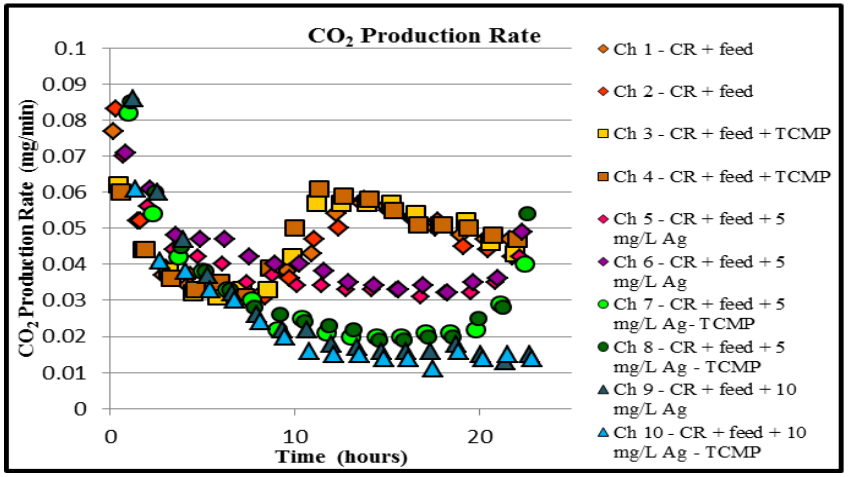


Figure B. 5. Raw data of Test 5 (CR - 18 October 2012) 5-10 mg/L Ag







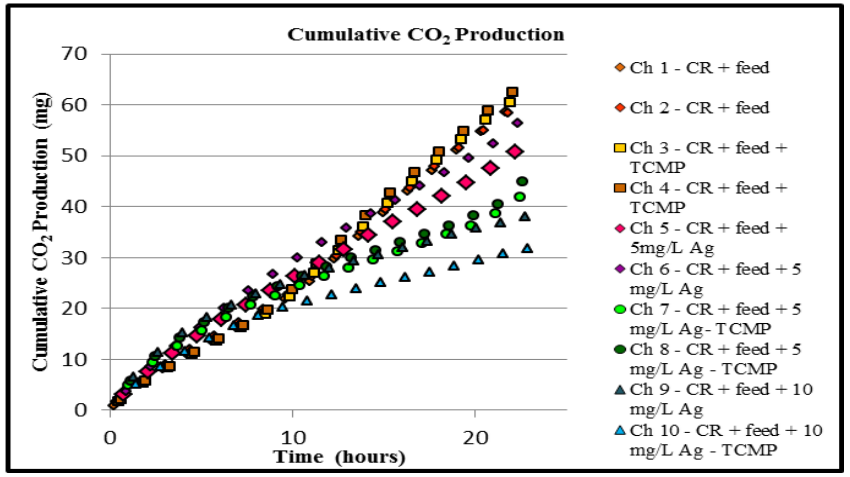


Figure B. 6. Raw data of Test 6 (CR - 22 October 2012) 5-10 mg/L Ag

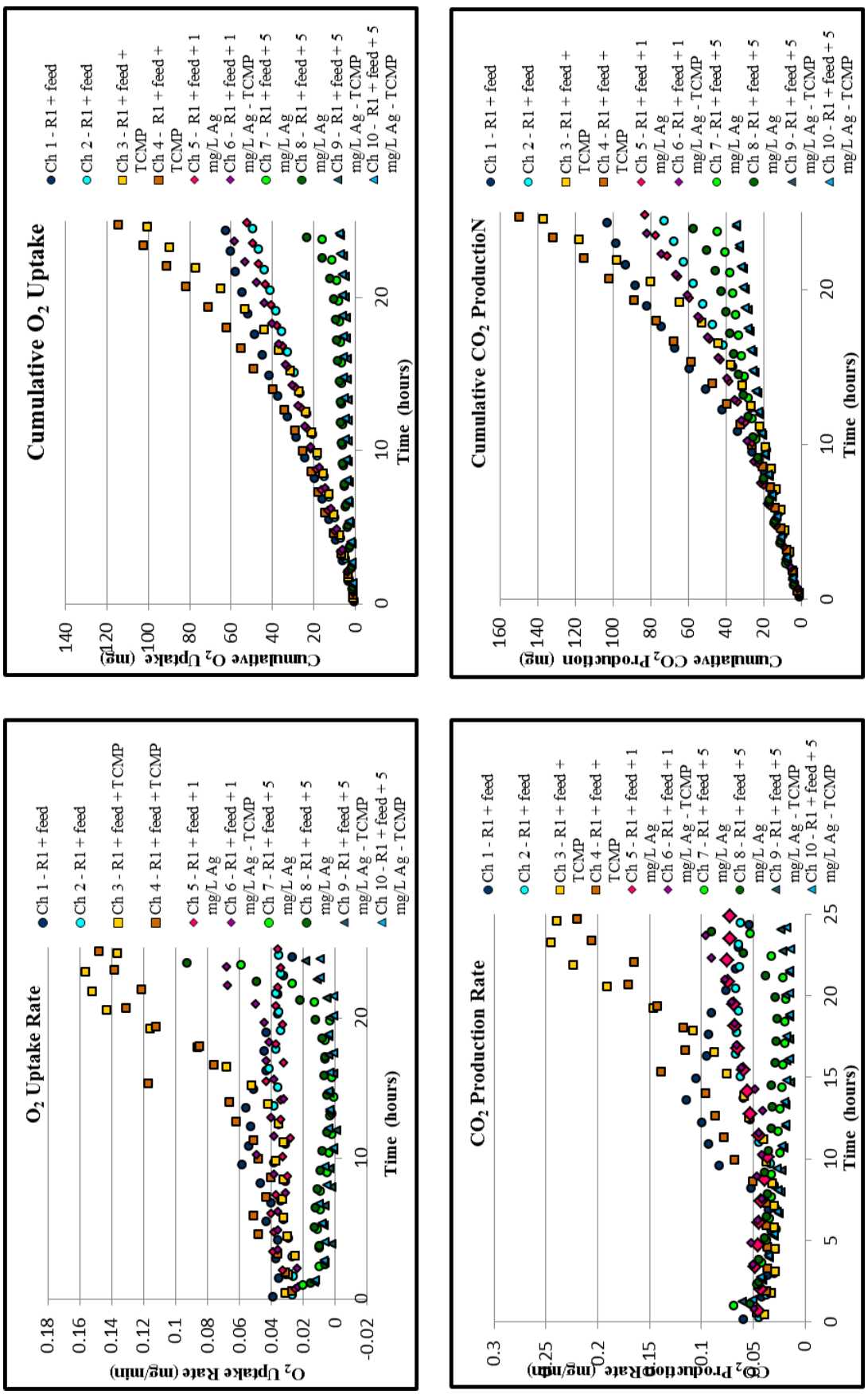
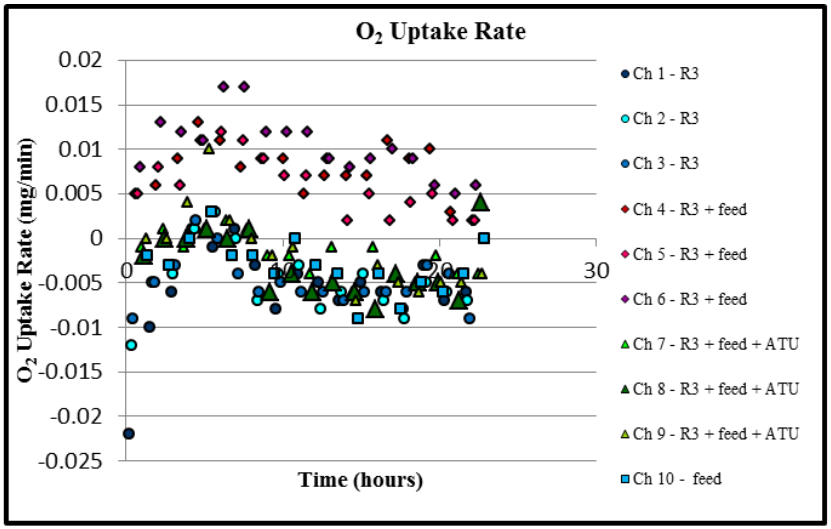
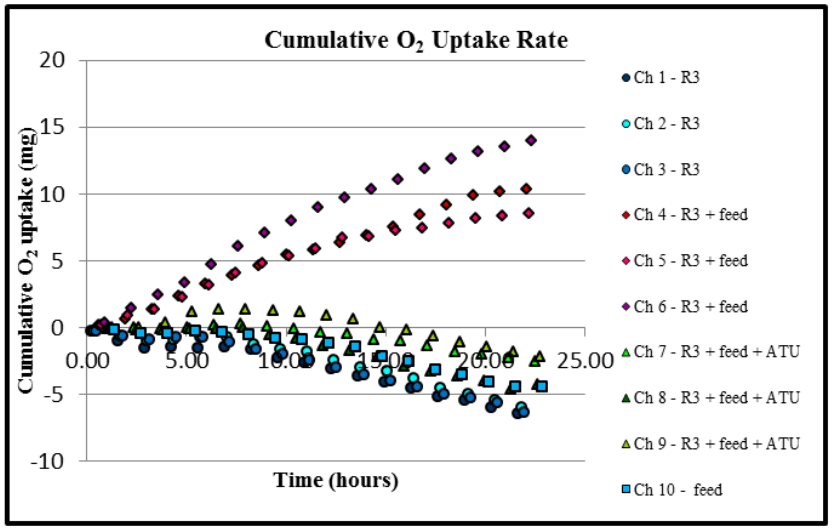
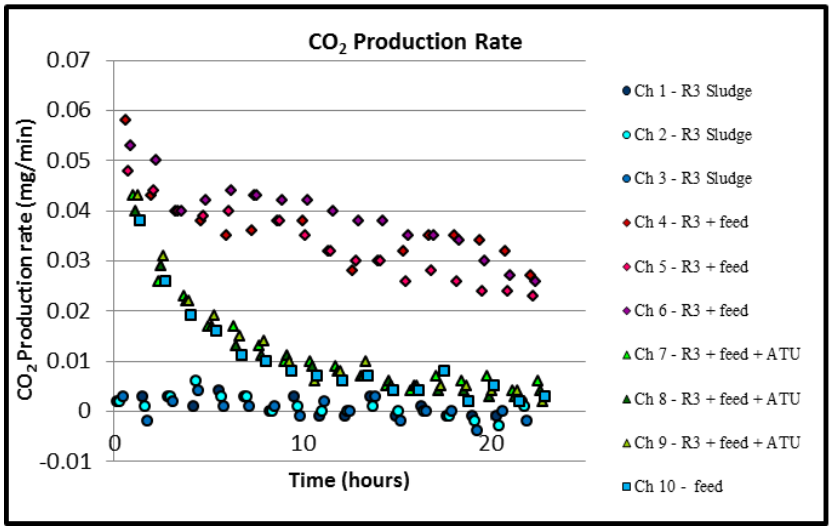


Figure B. 7. Raw data of Test 7 (R1 - 8 November 2012) 1-5 mg/L Ag







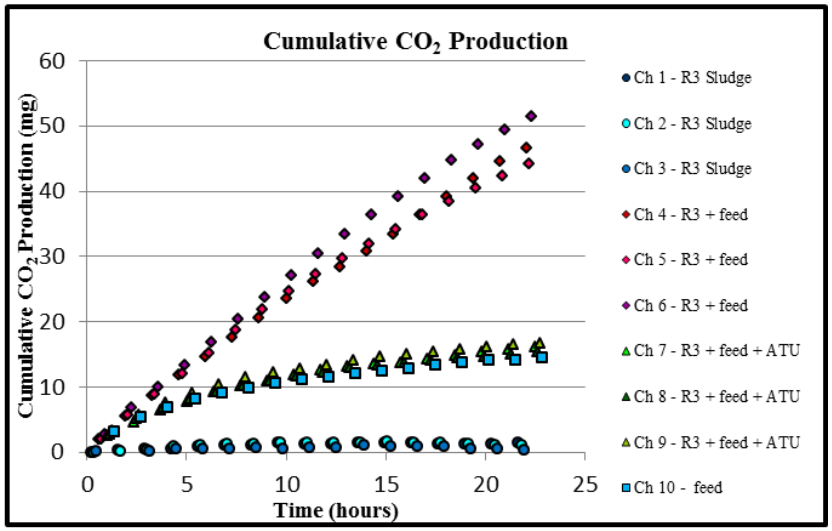


Figure B. 8. Raw data of Test 8 (R3 - 19 November 2012)

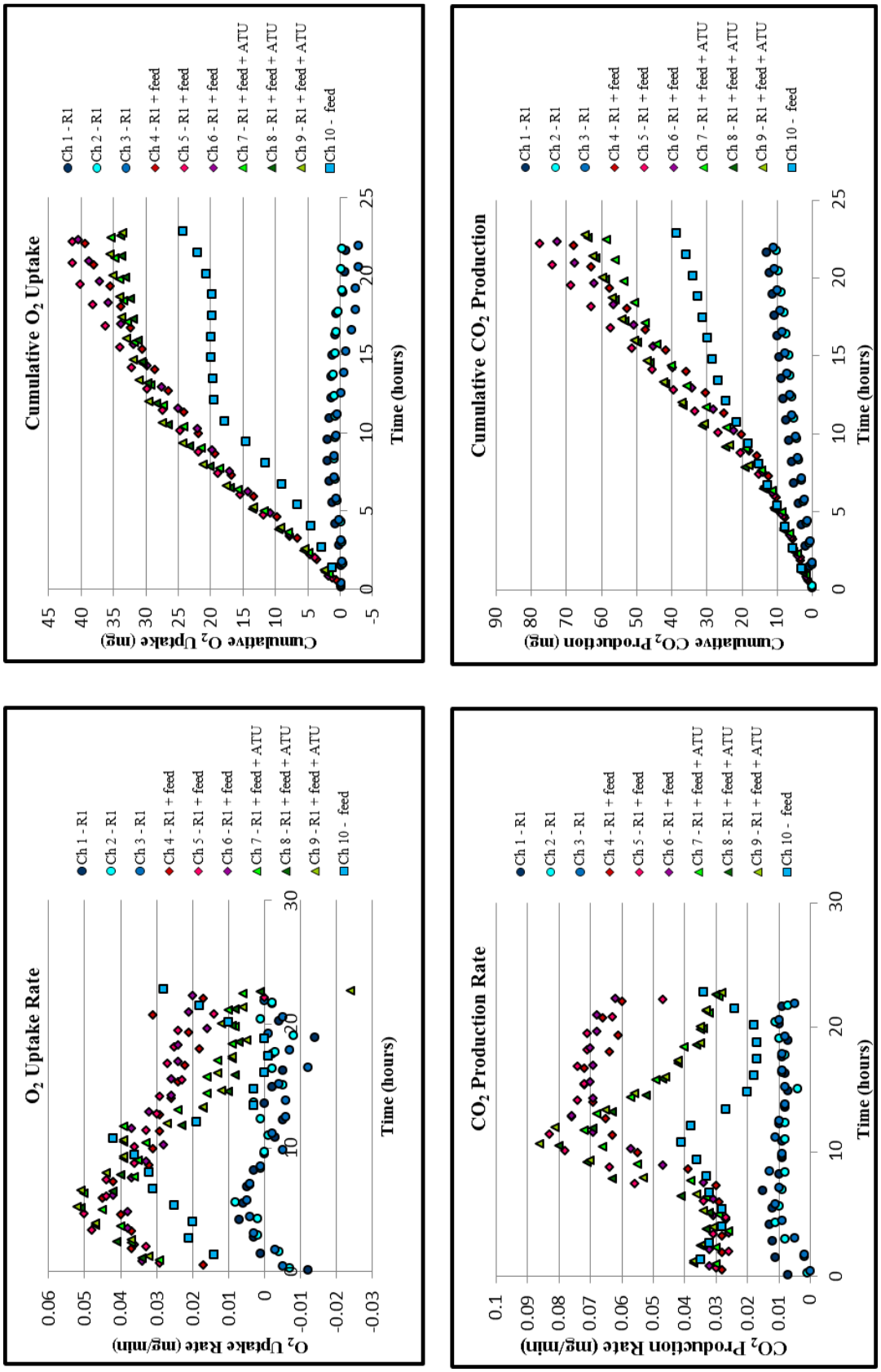


Figure B. 9. Raw data of Test 9 (R1 - 22 November 2012)

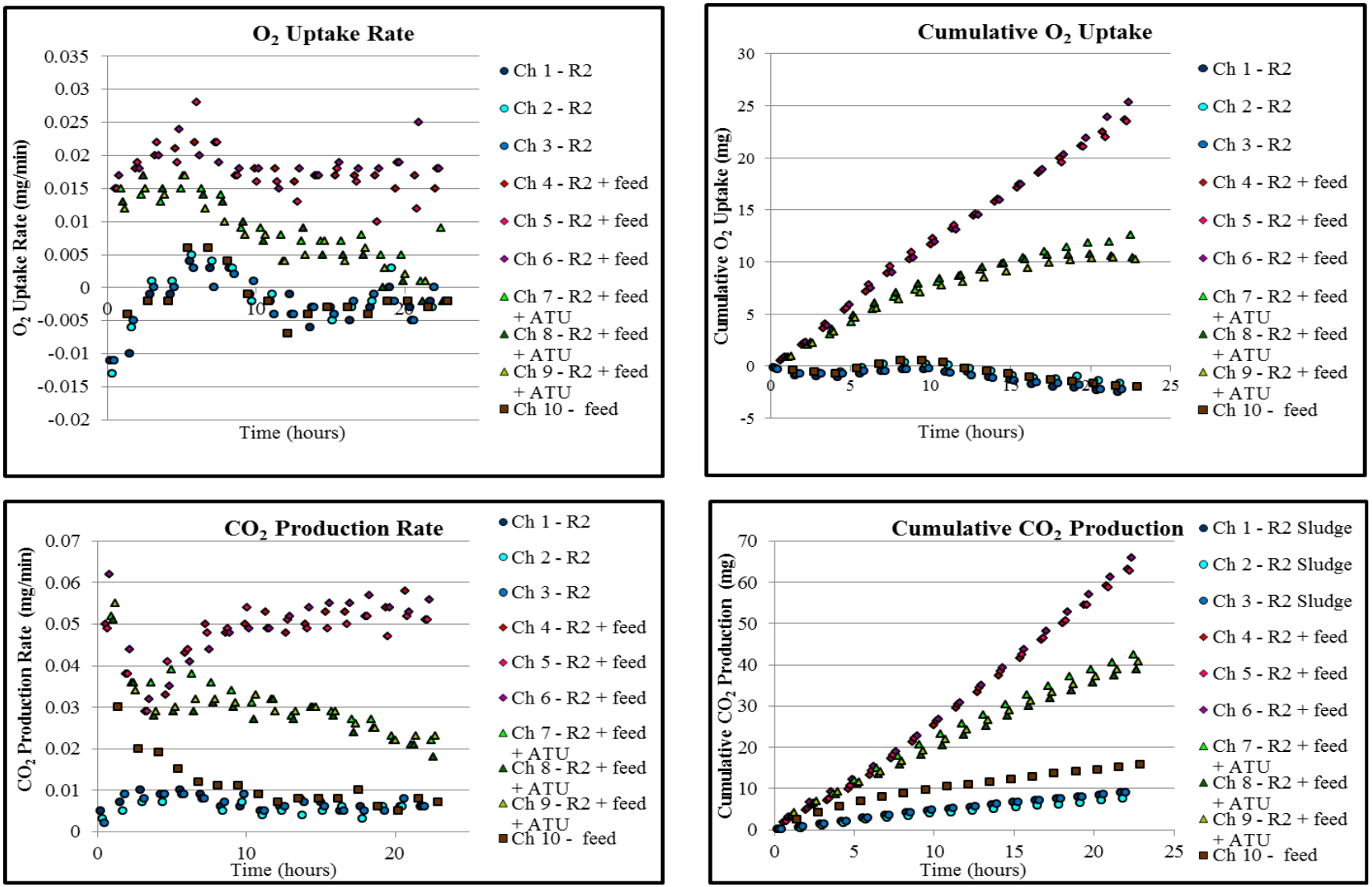


Figure B. 10. Raw data of Test 10 (R2 - 6 November 2012)

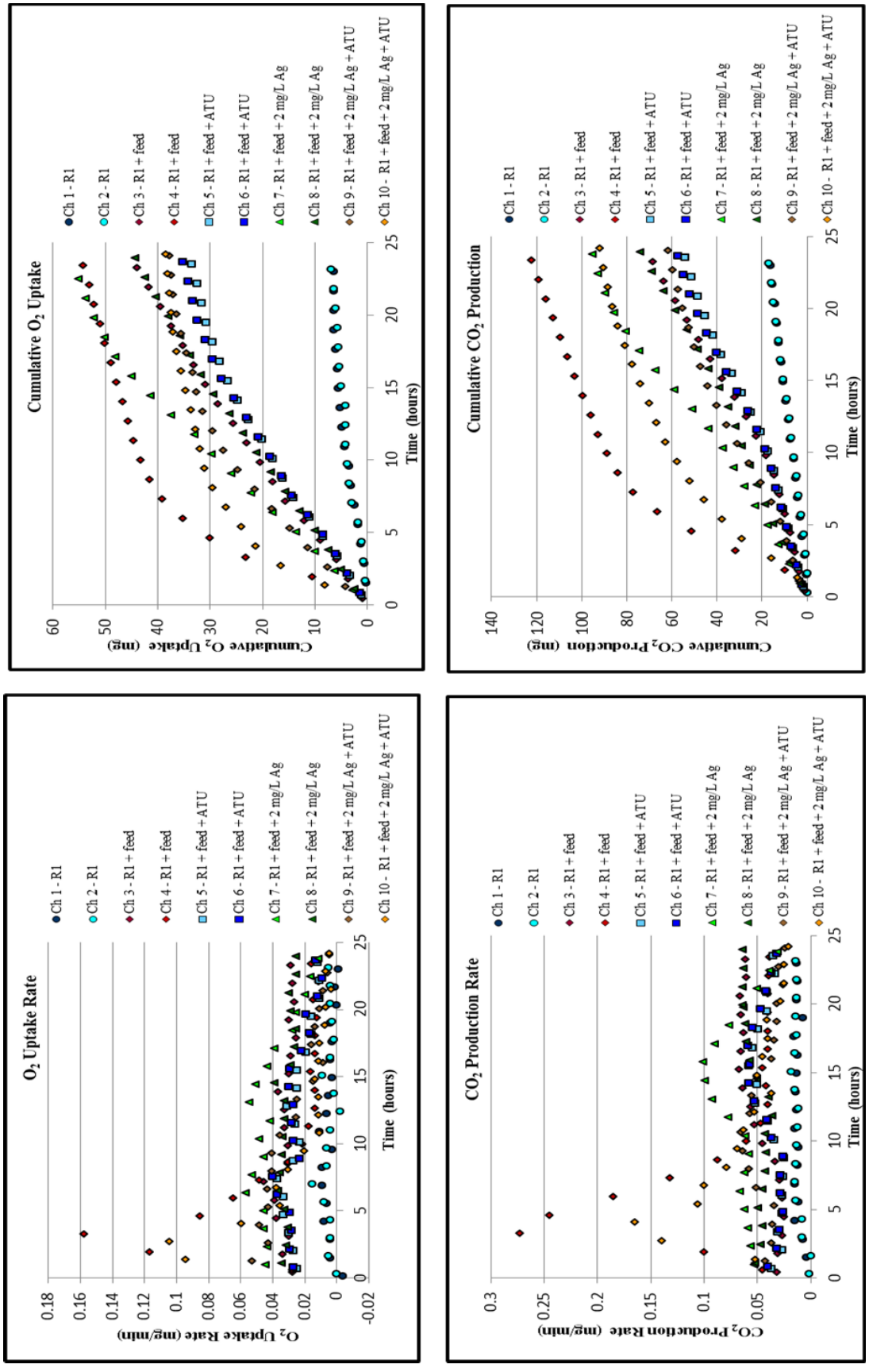


Figure B. 11. Raw data of Test 11 (R1 - 6 December 2012) 2 mg/L Ag

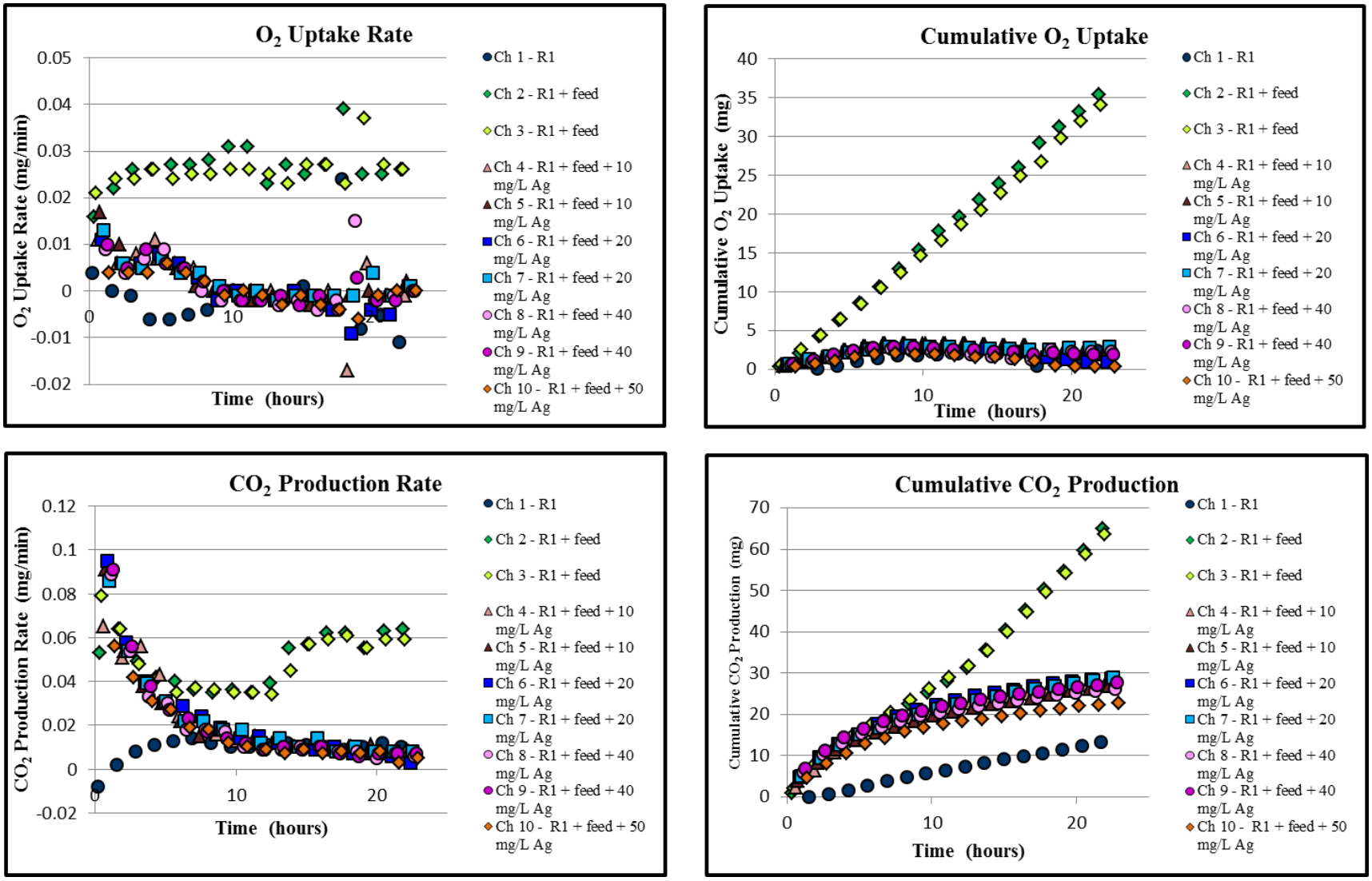


Figure B. 12. Raw data of Test 12 (R1 - 12 January 2013) 10-20-40-50 mg/L Ag

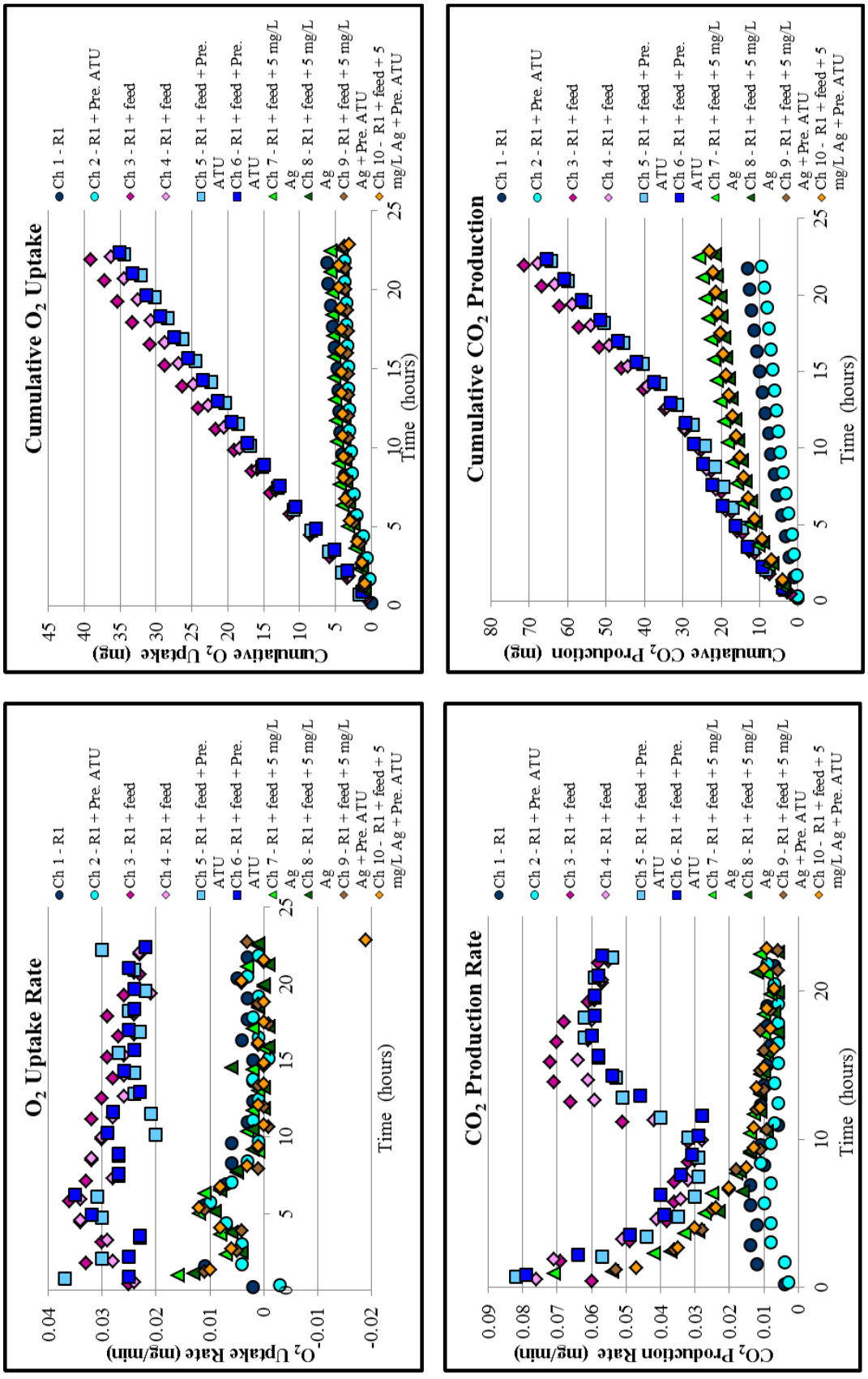


Figure B. 13. Raw data of Test 13 (R1 - 17 January 2013) 5 mg/L Ag

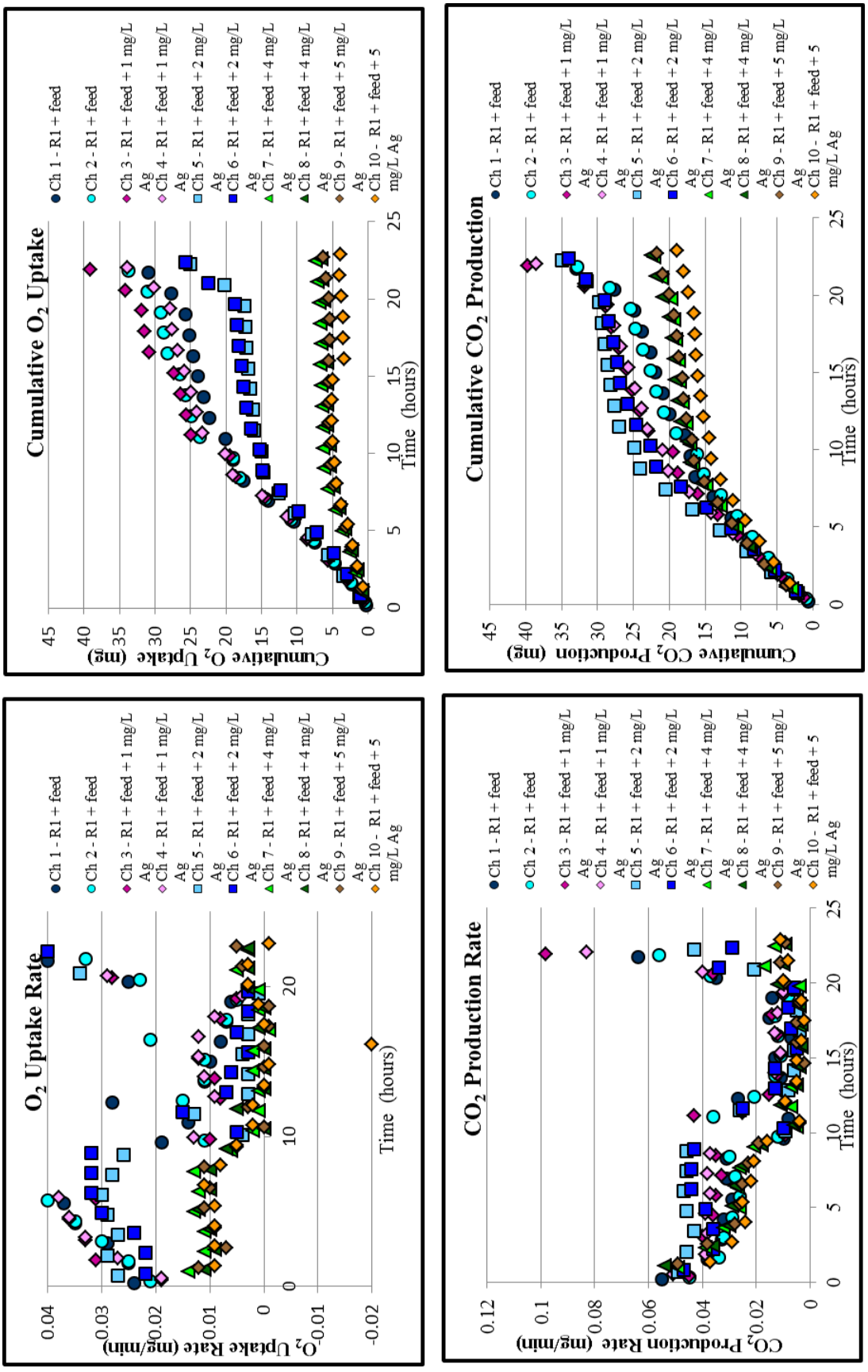


Figure B. 14. Raw data of Test 14 (R1 - 24 January 2013) 1-2-4-5 mg/L Ag

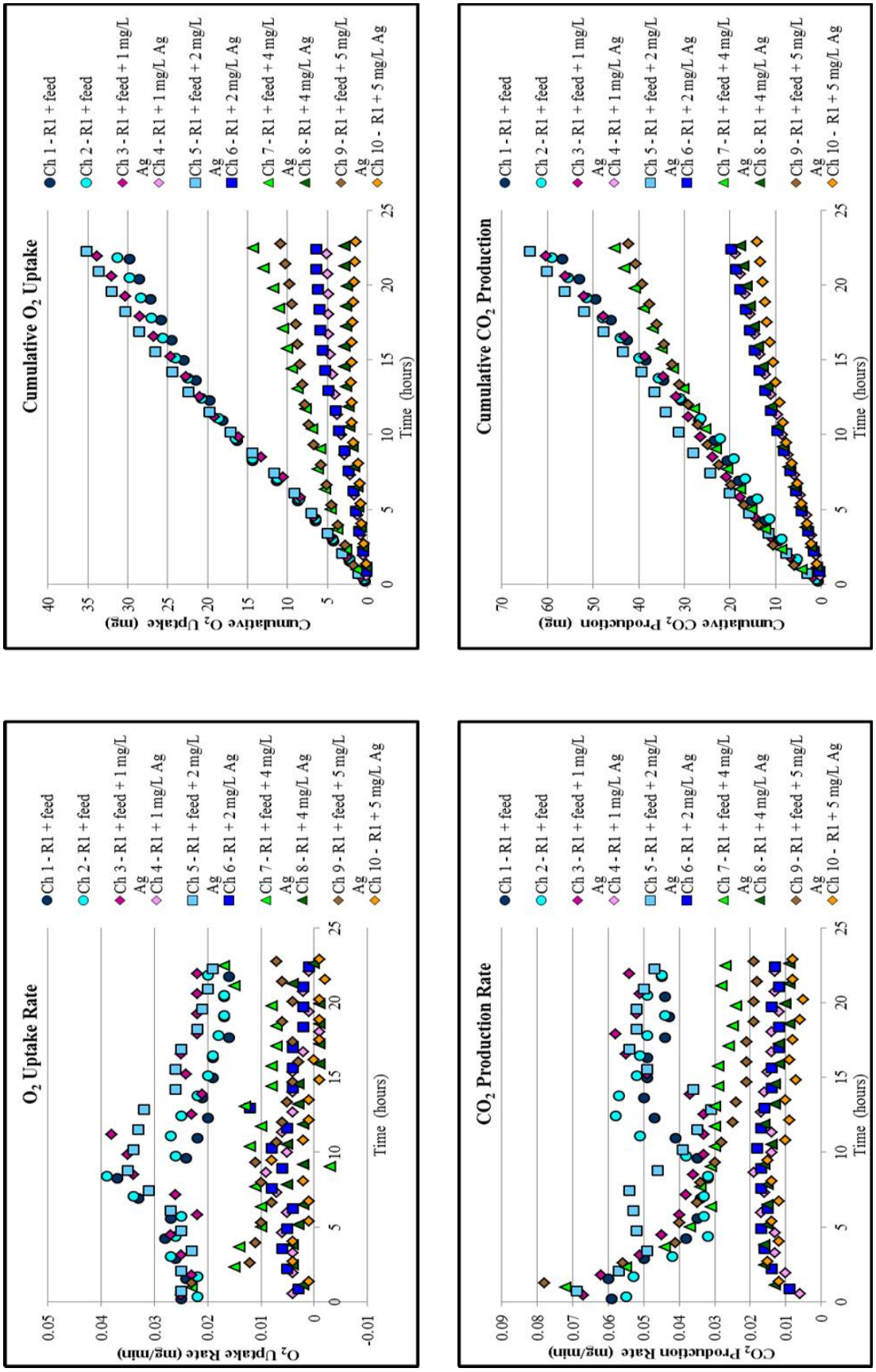


Figure B. 15. Raw data of Test 15 (R1 - 31 January 2013) 1-2-4-5 mg/L Ag

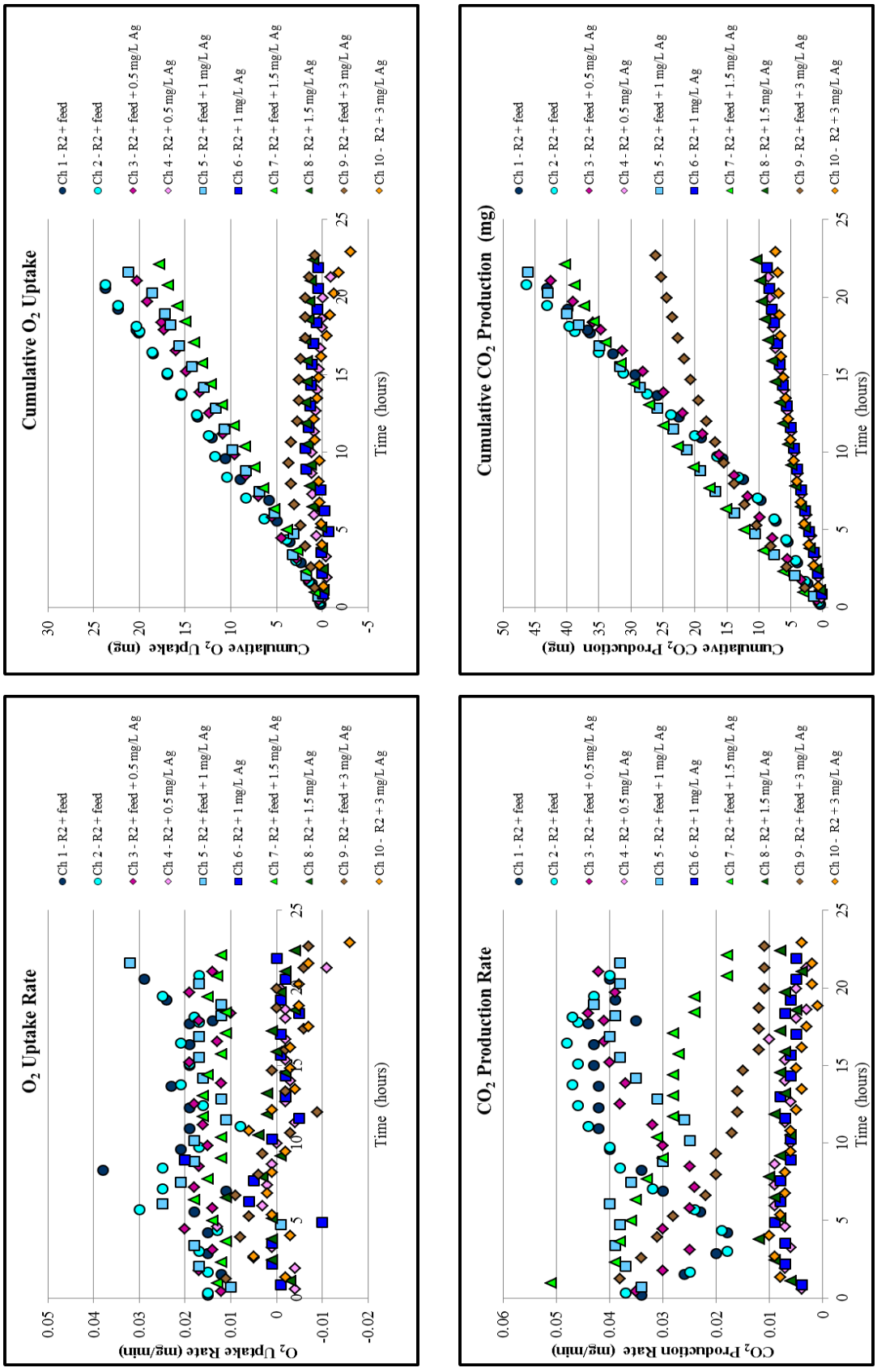


Figure B. 16. Raw data of Test 16 (R2 - 6 February 2013) 0.5-1-1.5-3 mg/L Ag

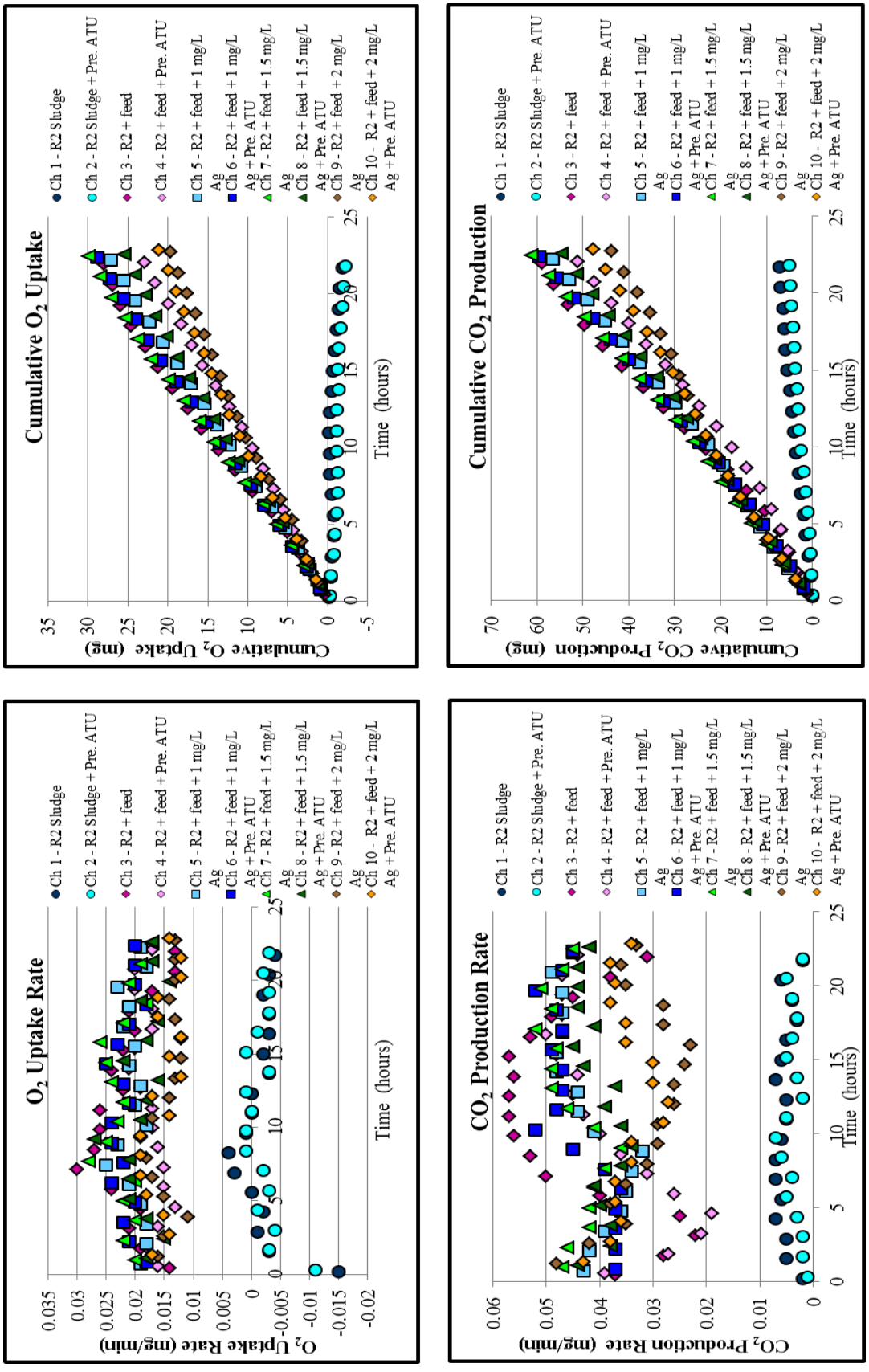


Figure B. 17. Raw data of Test 17 (R2 - 14 February 2013) 1-1.5-2 mg/L Ag

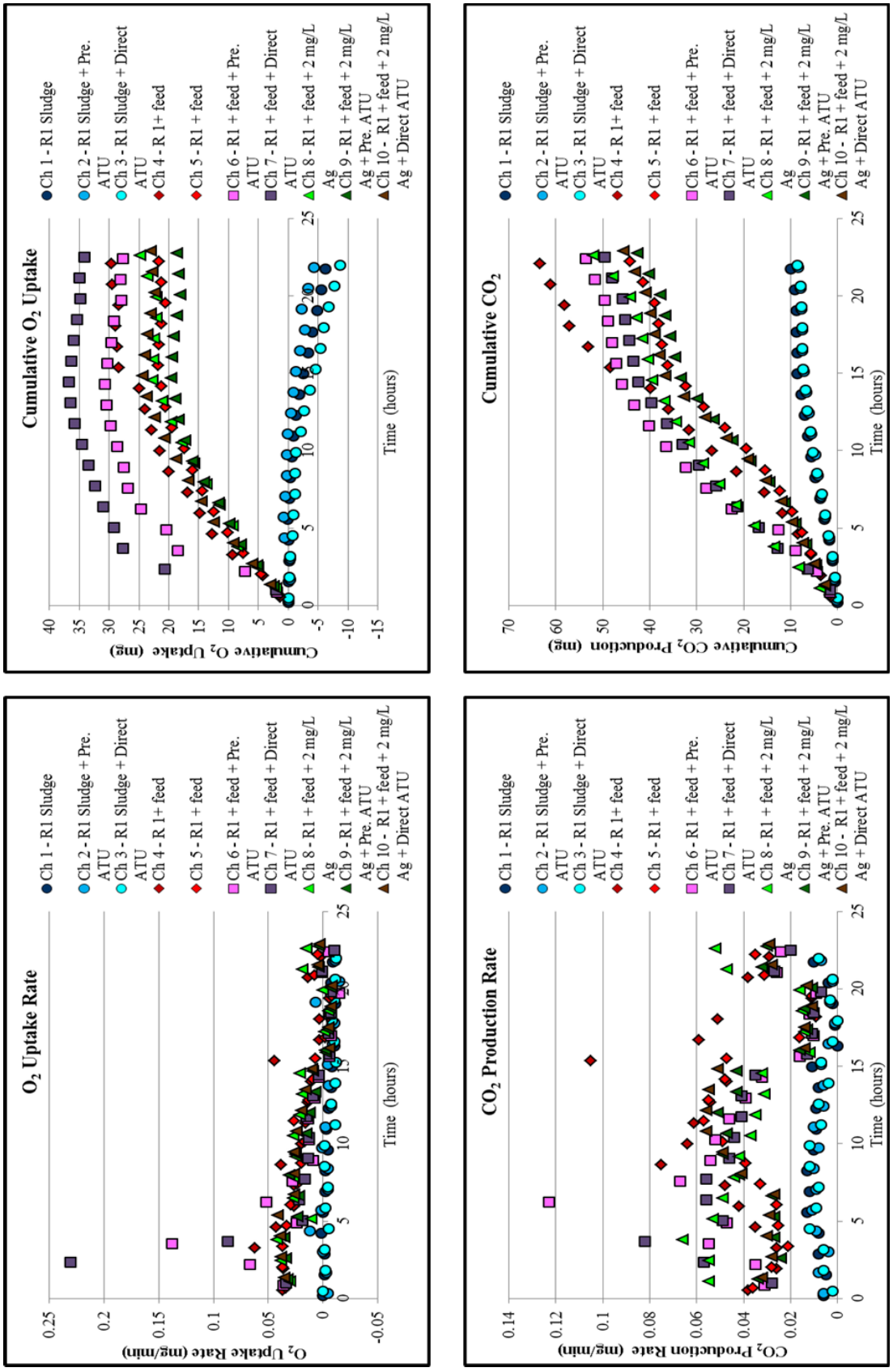


Figure B. 18. Raw data of Test 18 (R1 - 27 February 2013) 2 mg/L Ag

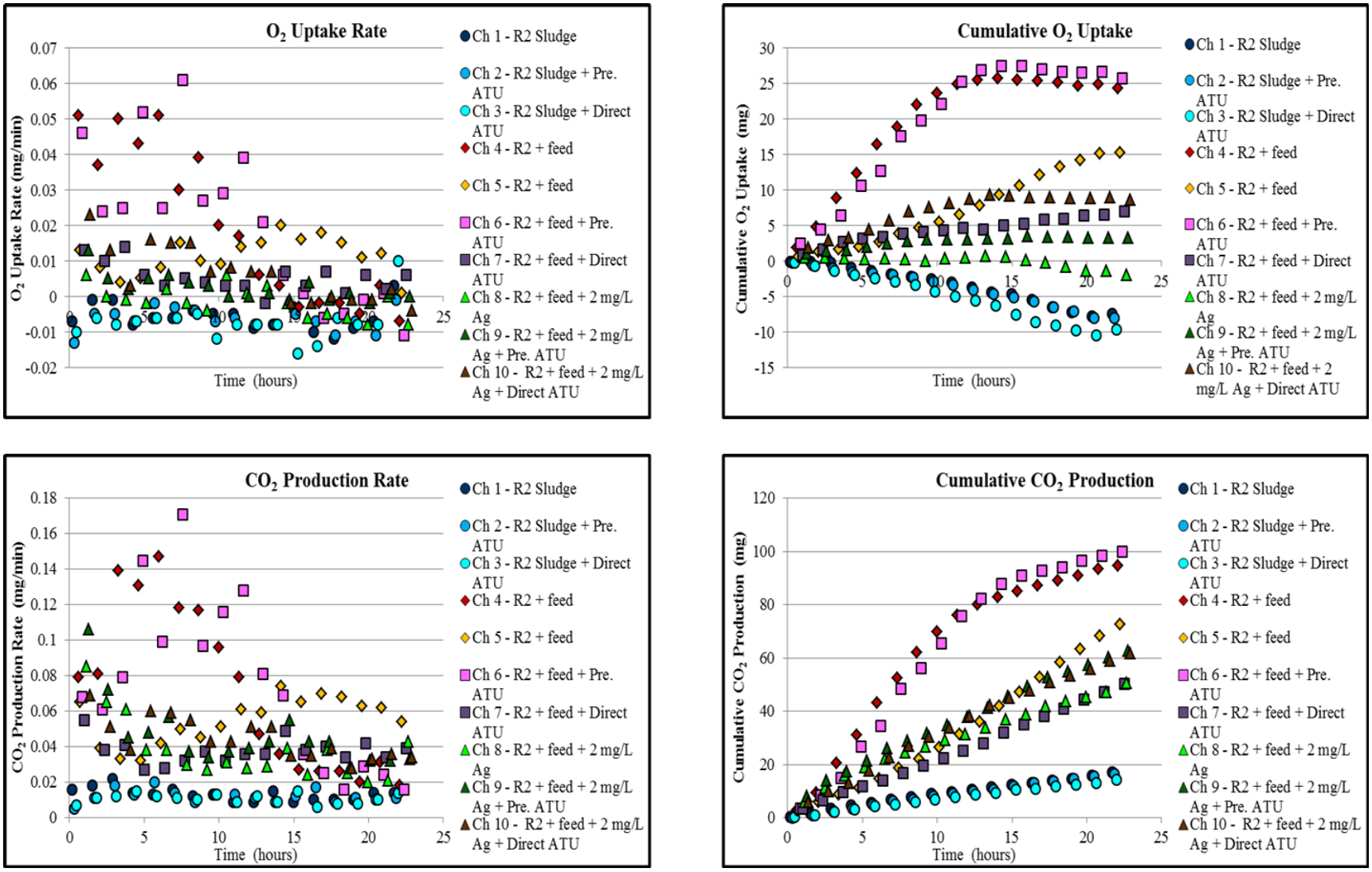


Figure B. 19. Raw data of Test 19 (R2 - 6 March 2013) 2 mg/L Ag

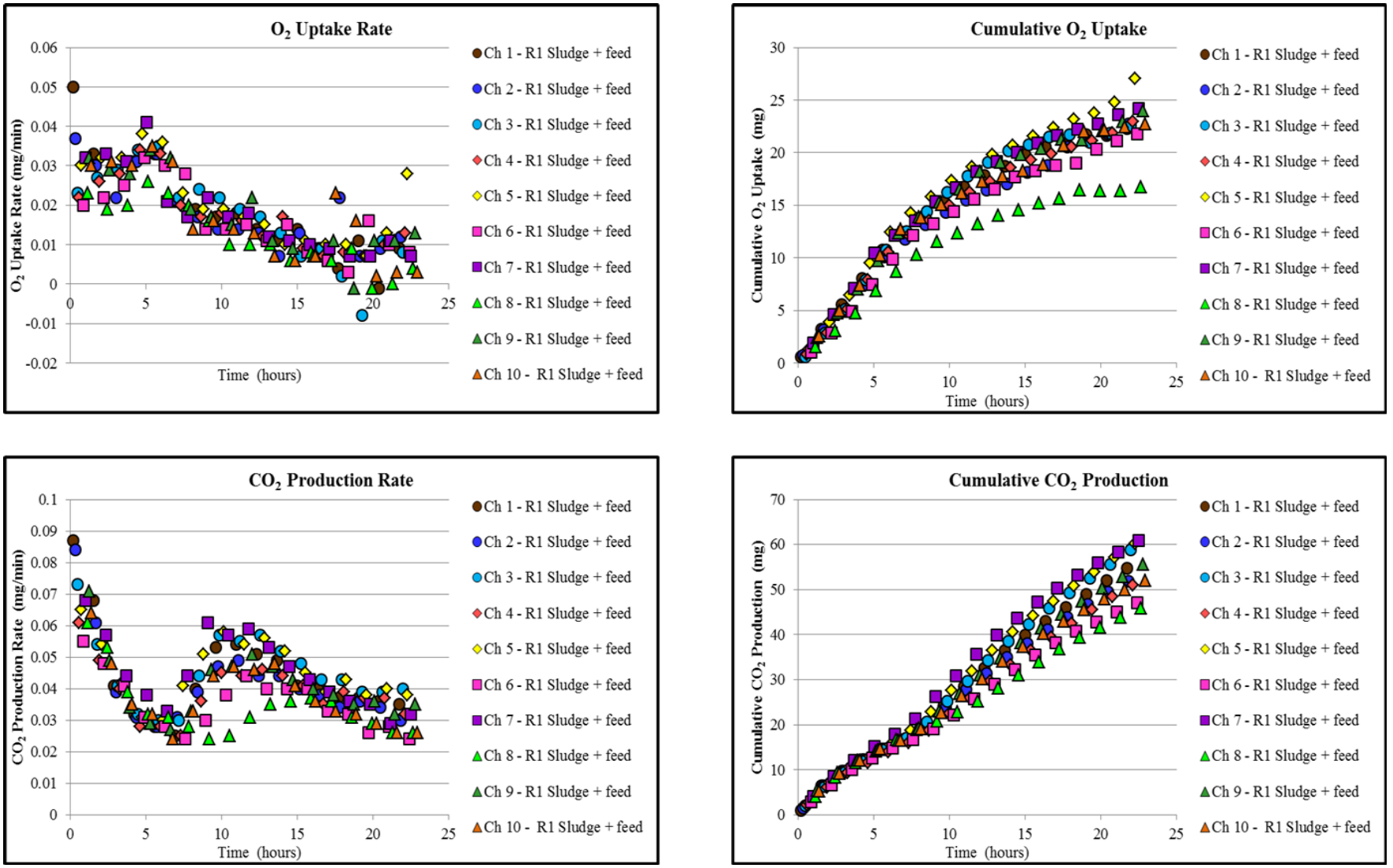


Figure B. 20. Raw data of Test 20 (R1 - 13 March 2013)

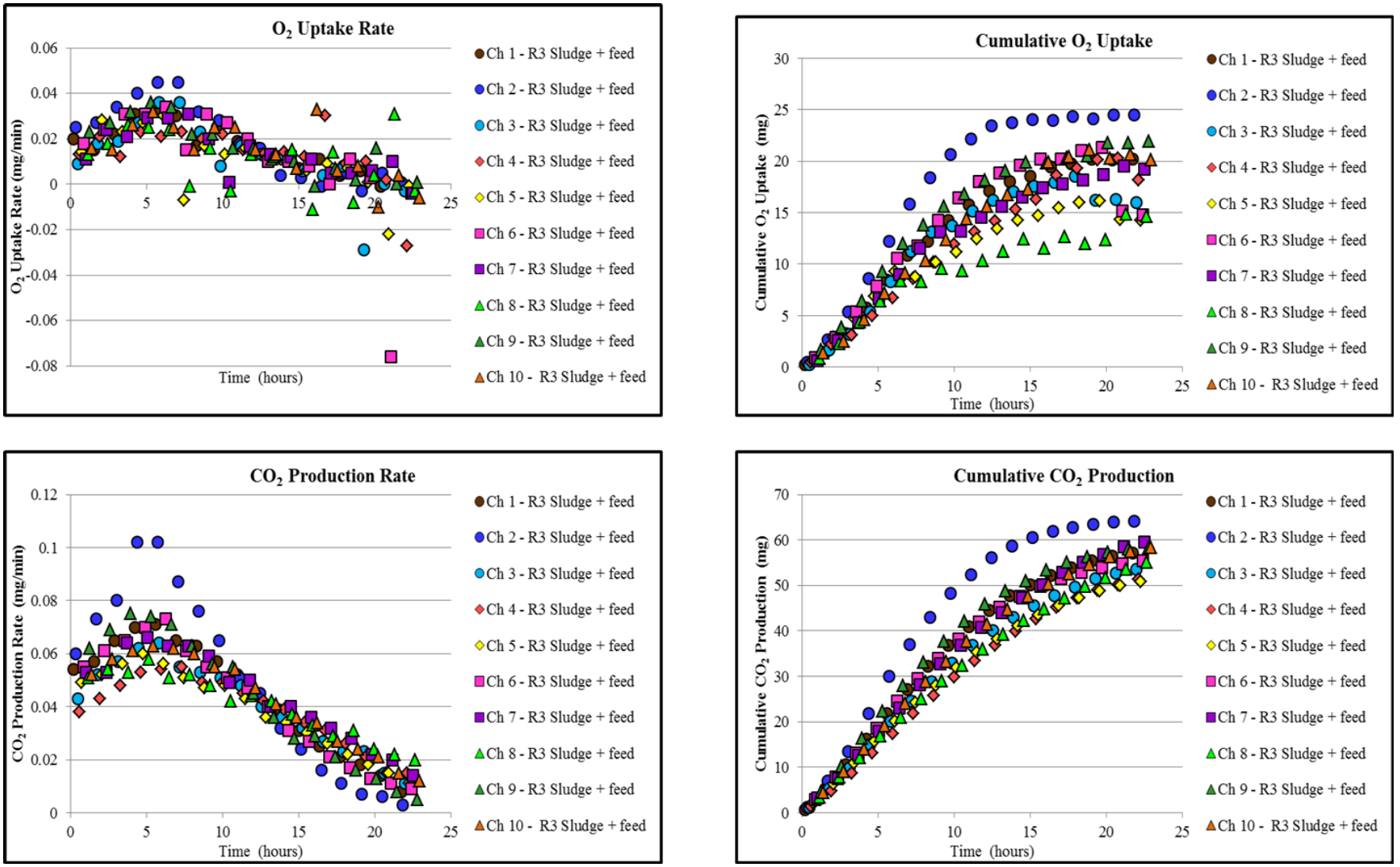


Figure B. 21. Raw data of Test 21 (R3 - 14 March 2013)

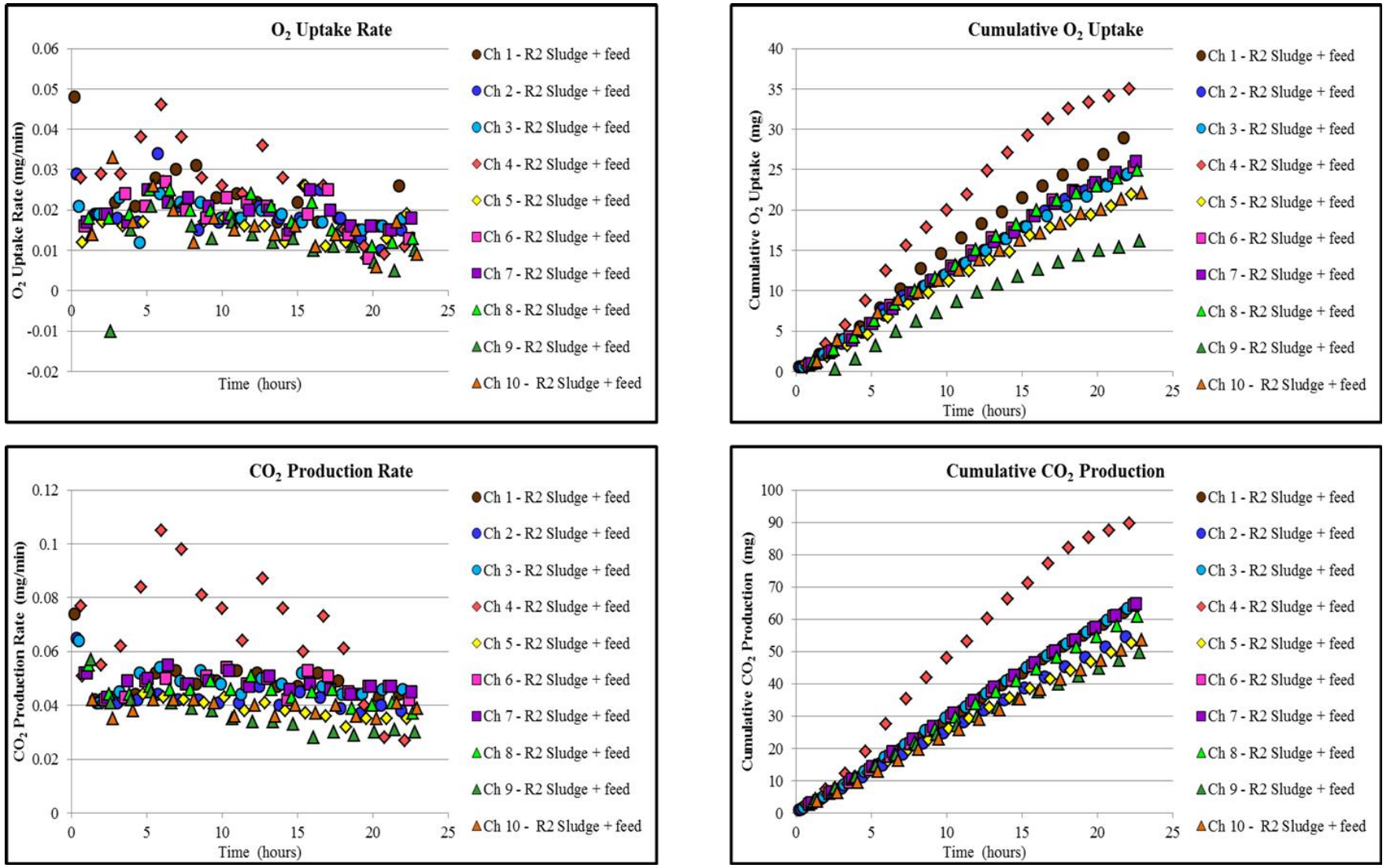


Figure B. 22. Raw data of Test 22 (R2 - 21 March 2013)

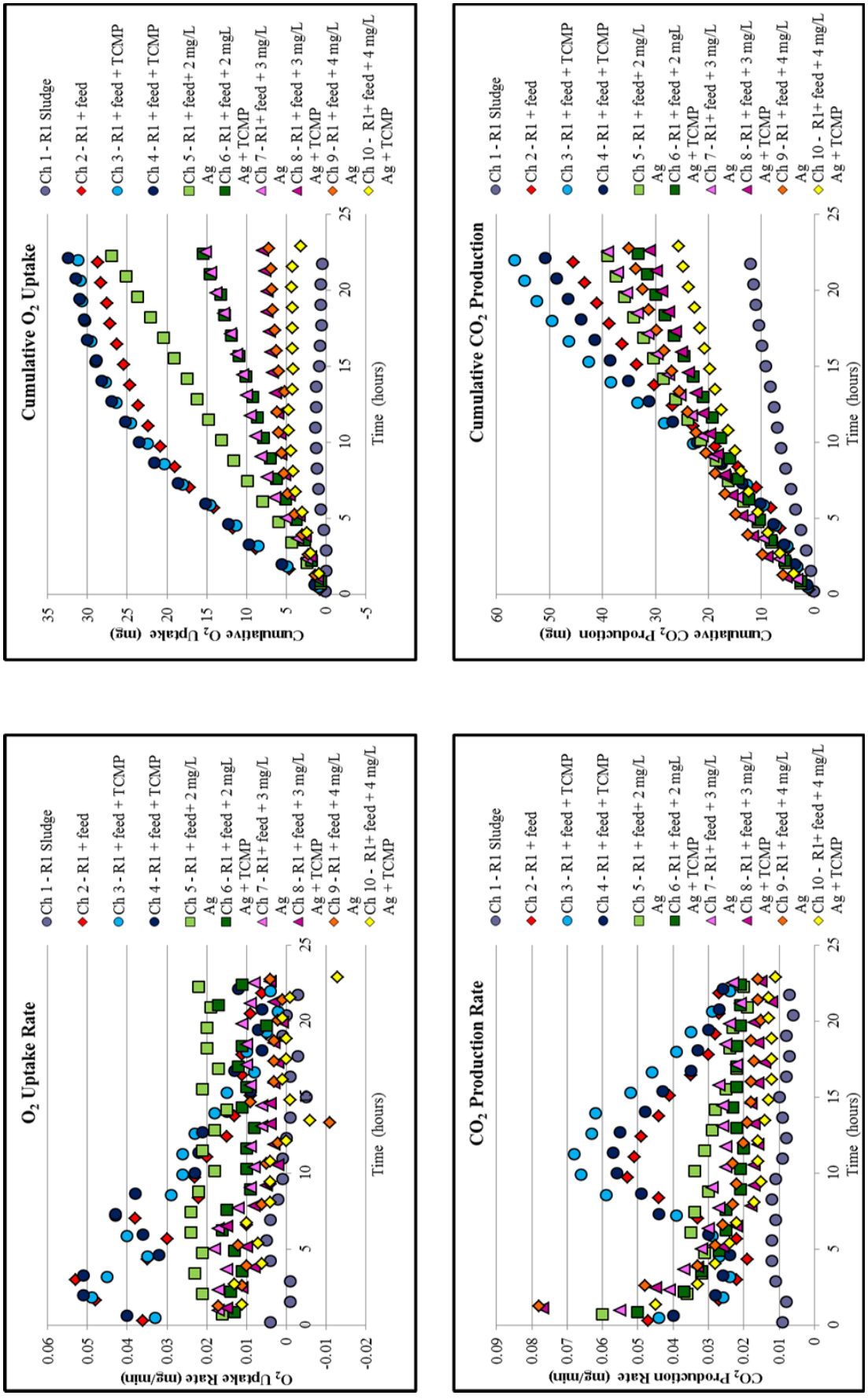


Figure B. 23. Raw data of Test 23 (R1 - 1 April 2013) 2-3-4 mg/L Ag

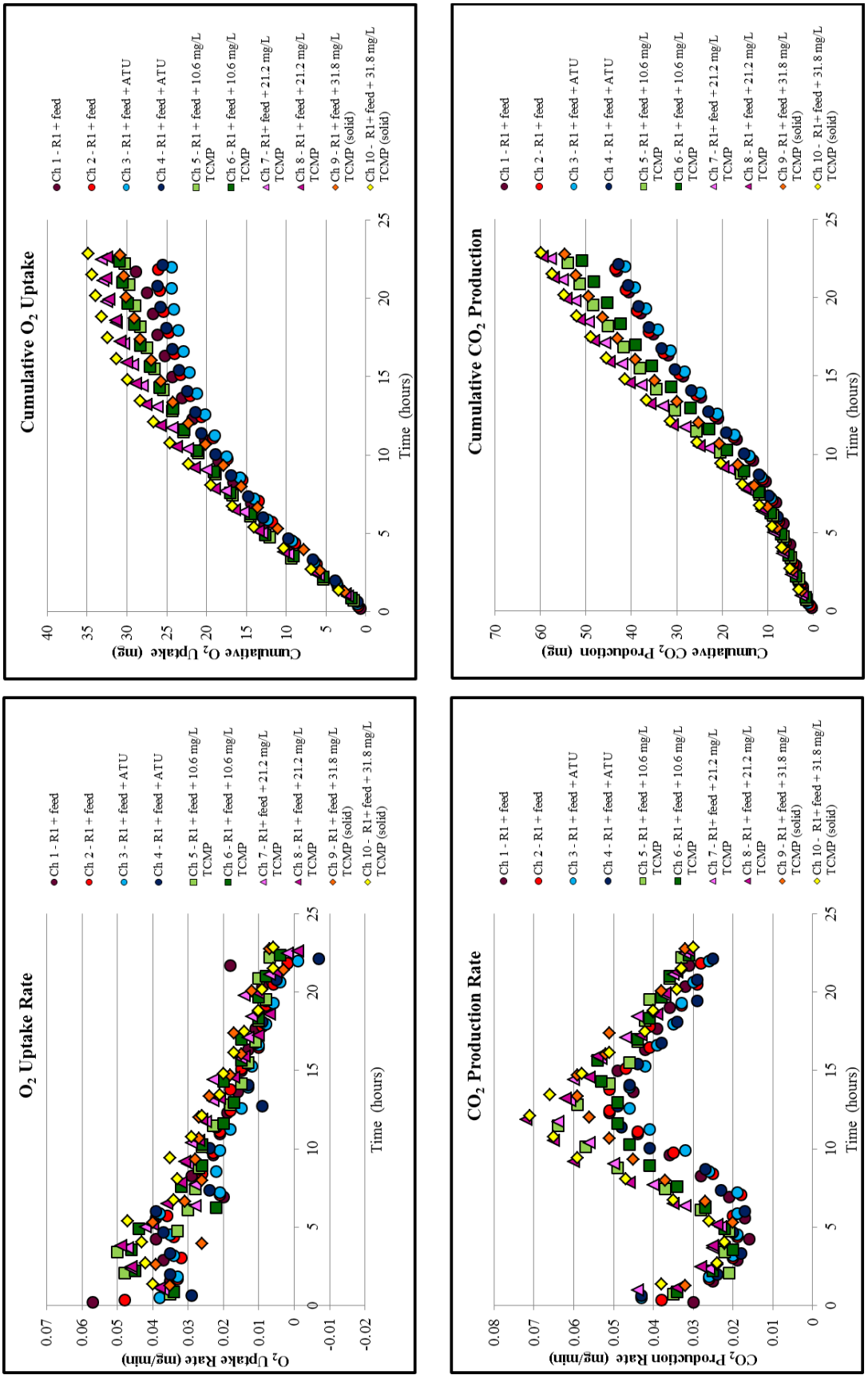


Figure B. 24. Raw data of Test 24 (R1 - 9 April 2013)

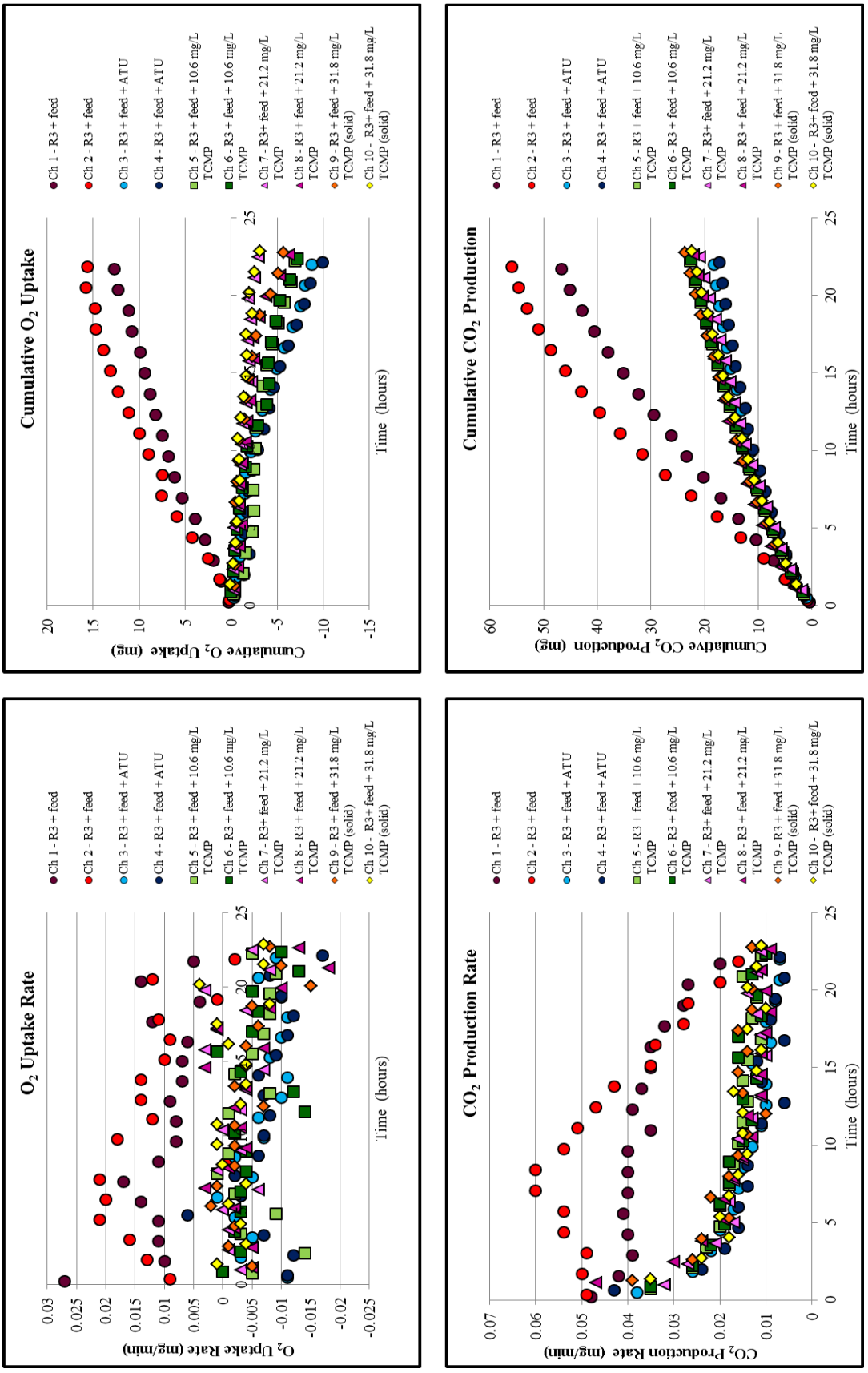


Figure B. 25. Raw data of Test 25 (R3 - 16 April 2013)

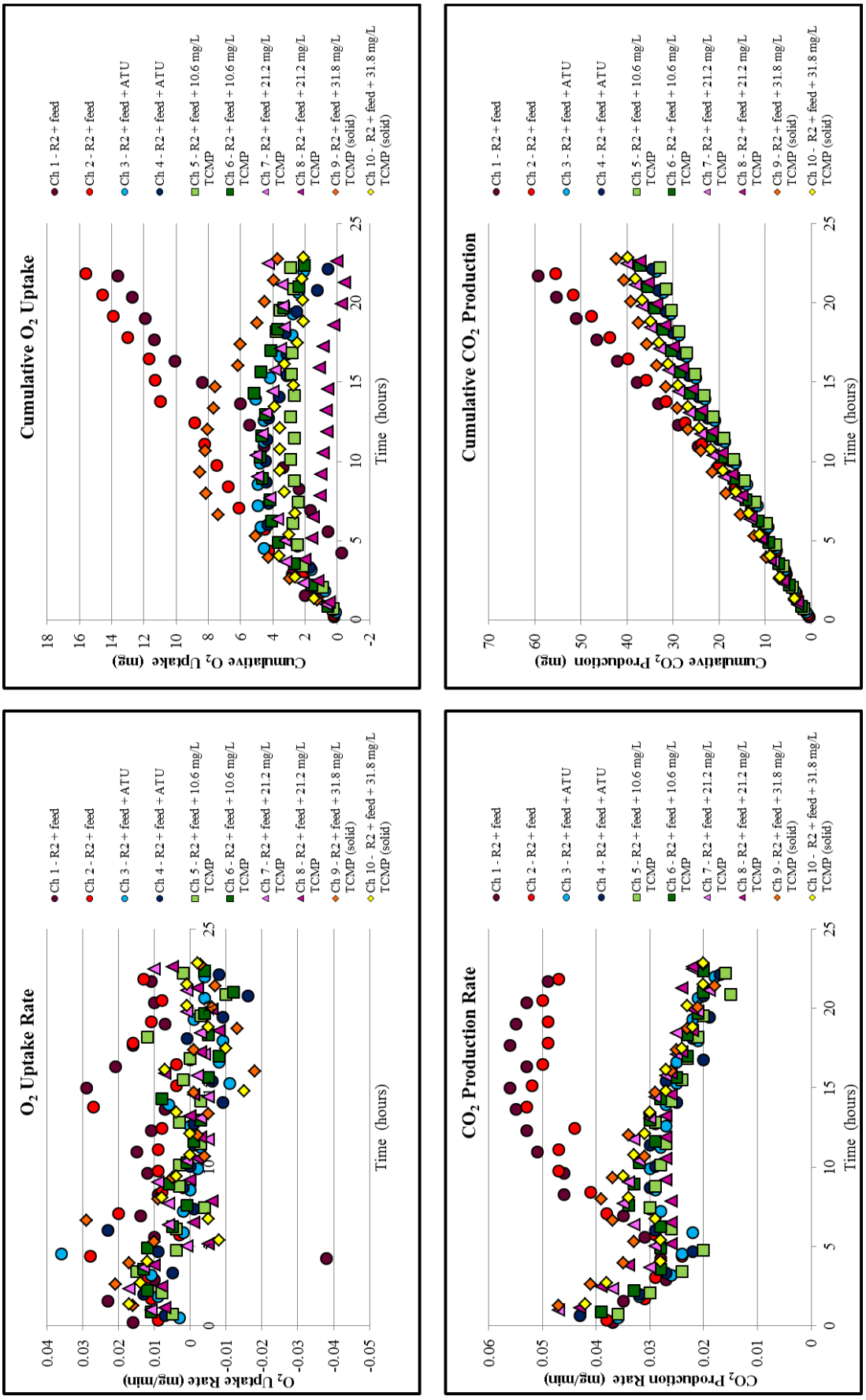


Figure B. 26. Raw data of Test 26 (R2 - 17 April 2013)