SILVER INHIBITION, SURFACE CHARGE AND HYDROPHOBICITY IN ACTIVATED SLUDGES FED WITH DIFFERENT SUBSTRATES

by

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ABSTRACT

SILVER INHIBITION, SURFACE CHARGE AND HYDROPHOBICITY IN ACTIVATED SLUDGES FED WITH DIFFERENT SUBSTRATES

Silver is one of heavy metals which is used in many industries. It affects the performance of biological treatment plants because of its toxic effect on microorganisms.

The objective of this study is to determine the effect of silver on activated sludges fed with different substrates (a mixture consisting of glucose, peptone and sodium acetate, glucose only, peptone only). For this purpose, three semi-continuously fed batch reactors were operated for 438 days at steady-state condition. The inhibitory effect of silver was examined with the results of O_2 and CO_2 measurements in respirometric tests. Additionally, differences between surface charges and hydrophobicities of sludges were examined by using the colloidal titration and microbial adhesion to hydrocarbons methods, respectively.

Results of the study showed that feed composition has a role on the inhibitory effect of silver ion. The sludges fed with mixed substrates and only glucose were highly affected at 4 and 5 mg/L silver addition, while the sludge fed with only peptone could tolerate these silver concentrations. This indicated that peptone reduces inhibitory effect of silver. In addition, sludges fed with mixed substrates and only glucose had higher surface charges and lower hydrophobicities compared to the sludge fed with only peptone.

ÖZET

FARKLI SÜBSTRATLARLA BESLENEN AKTİF ÇAMURLARDA YÜZEY YÜKÜ, HİDROFOBİSİTE VE GÜMÜŞ METALİNİN İNHİBİSYON ETKİSİ

Gümüş, pek çok endüstride kullanılan bir ağır metaldir. Gümüş, mikroorganizmalar üzerindeki toksik etkisi sebebiyle biyolojik atıksu arıtma tesislerinin performansını etkiler.

Bu çalışmanın amacı, gümüşün farklı sübstratlarla (glikoz, pepton ve sodyum asetat içeren besi, sadece glikoz içeren besi, sadece pepton içeren besi) beslenen aktif çamurlar üzerindeki etkisini belirlemektir. Bu amaçla, üç farklı reaktör kararlı halde yarı-kesikli beslenerek 438 gün boyunca işletilmiştir. Gümüş iyonunun inhibisyon etkisi respirometrik testlerdeki O_2 ve CO_2 ölçümleri kullanılarak belirlenmiştir. Buna ek olarak, aktif çamurların yüzey yükü ve hidrofobisiteleri arasındaki farklılıklar da kolloidal titrasyon ve microorganizmaların hidrokarbonlara tutunma kapasitesi metotları kullanılarak tetkik edilmiştir.

Bu çalışmanın sonuçları, besi kompozisyonunun gümüşün inhibisyon etkisi üzerinde rolü olduğunu göstermiştir. Karışık sübstrat ve sadece glikoz ile beslenen aktif çamurlar 4 ve 5 mg/L gümüş konsantrasyonundan oldukça etkilenirken, sadece pepton ile beslenen aktif çamurun bu konsantrasyonları tolere edebildiği saptanmıştır. Buna göre, peptonun gümüşün inhibisyon etkisini azalttığı belirlenmiştir. Buna ek olarak, karışık sübstrat ve sadece glikoz ile beslenen aktif çamurların, sadece pepton ile beslenen aktif çamura göre daha yüksek yüzey yükü ve daha düşük hidrofobisiteye sahip oldukları gözlenmiştir.

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LIST OF SYMBOLS / ABBREVIATIONS

Symbol	Explanation	Units used
μ	Specific Growth Rate	(1/d)
Ag^+	Free silver ion	(mg/L)
AOB	Ammonia Oxidizing Bacteria	
ATU	N-Allylthiourea	(mg/L)
b	Decay coefficient of microorganisms	(1/d)
BOD	Biochemical Oxygen Demand	(mg/L)
C/N	Carbon to Nitrogen ratio	
C-CO_2	Carbonaceous CO_2 production	(mg)
C-O_2	Carbonaceous oxygen uptake	(mg)
COD	Chemical Oxygen Demand	(mg/L)
CR	Control Reactor	
DO	Dissolved Oxygen	(mg/L)
EPS	Extracellular Polymeric Substances	
F/M	Food to Microorganism Ratio	
Gch	Glycolchitosan	
K	Half saturation constant	(mg/L)
LB-EPS	Loosely Bound-Extracellular Polymeric Substances	
MATH	Microbial adhesion to hydrocarbons test	
MLSS	Mixed Liquor Suspended Solids	(mg/L)
MLVSS	Mixed Liquor Volatile Suspended Solids	(mg/L)
N-CO_2	Nitrogenous CO_2 production	(mg)
NH_4-N	Ammonium nitrogen	(mg/L)
N-O_2	Nitrogenous oxygen uptake	(mg)
NO_2-N	Nitrite nitrogen	(mg/L)
NO_3-N	Nitrate nitrogen	(mg/L)
NOB	Nitrite Oxidizing Bacteria	
OUR	Oxygen Uptake Rate	(mg/L.h)
POTW	Publicly Owned Treatment Works	

PVSK	Potassium poly(vinly)sulfate	
q	Specific Substrate Removal Rate	
R1	Reactor 1	
R2	Reactor 2	
R3	Reactor 3	
RG	Glucose Reactor	
RP	Peptone Reactor	
SC	Surface Charge	(meqv/g MLSS)
SCFB	Semi-Continuously Fed Batch (Reactor)	
SOUR	Specific Oxygen Uptake Rate	(mg/L.h.mg)
SRT	Solids Retention Time	(d)
SS	Suspended Solids	(mg/L)
TB	Toluidine Blue	
TB-EPS	Tightly Bound-Extracellular Polymeric Substances	
T-CO_2	Total CO_2 production	(mg)
TKN	Total Kjeldahl Nitrogen	(mg/L)
T-O_2	Total oxygen uptake	(mg)
VSS	Volatile Suspended Solids	(mg/L)
X_a	Active biomass concentration	(mg/L)
Y	Yield coefficient	(mg/mg)

1. INTRODUCTION

In recent years, heavy metal pollution has become one of the important environmental problems. Heavy metals even in traces are toxic and dangerous to both fauna and flora. The increase in industrial activities causes environmental pollution with the accumulation of heavy metals. Heavy metals are very toxic and accumulate throughout the food chain. Wastes which contain metals are directly or indirectly discharged into the environment and cause environmental pollution and threat human life (Das et al., 2008; Yuncu et al., 2006). Heavy metals may enter aquatic systems from industrial effluents, landfill leachates and municipal wastewaters. The inhibitory effects of heavy metals may show great variations in natural, contaminated, or man-made systems depending on the type and concentration (Çeçen et al., 2010).

Activated sludge is a biological treatment process that is commonly used for the removal of colloidal and soluble organic matter present in wastewater. The main role of municipal treatment plants is to remove soluble and colloidal organic matter. However, metals are also frequently present in municipal sewage (Oviedo et al., 2002). It is stated that toxic chemicals in the wastewater can inhibit some enzymes of the pathways in anabolism or catabolism. This causes inhibition of respiration and biodegradation. Heavy metals may also change the microbial structure of activated sludge and have negative effects on the growth and survival of microorganisms. As a result, heavy metals lower the effectiveness of biological processes in wastewater treatment plants (Hartmann et al., 2013). The harmful effects of heavy metals on biological processes are complex and generally depend on the type and the solubility of metal, characteristics of the influent and the concentration of the toxic material (Oviedo et al., 2002).

Silver is a heavy metal that is widely used in recent years. It is used in cosmetics, washing machines, cleaners, food containers, electroplating industry as a protective coating and photographing industry (Choi and Hu, 2009; Chen and Ray, 2001). It is thought that the free silver ion is the most toxic silver species. The inhibitory effect of free silver ion comes

from its sorption to negatively charged bacterial cell wall which causes deactivation of cellular enzymes (Choi et al., 2008).

The effect of metals in activated sludge is often attributed to the binding of metals on bacterial cell surface. Bacteria produce macromolecules outside their cell wall which are called extracellular polymeric substances (EPS). These molecules serve as a protective barrier for cells against the harsh external environment. Their composition is complex, but they are mainly composed of polysaccharides, humic substances, proteins, uronic acids, nucleic acids and lipids. They have ionisable functional groups which represent potential binding sites for the sequestration of metal ions (Comte et al., 2007). EPS play an important role in degradation of particulate substances and sorption of dissolved heavy metals. The electrostatic interactions between negatively charged biopolymeric substances outside the cells and metals lead to formation of stable complexes. EPS outside the cells are able to chelate some metals and bind them to cell surface. As a result, EPS protect cells from heavy metal stress (Pal and Paul, 2008).

It is mentioned that most EPS are anionic and nonionic in nature and this property determines the surface charge and selectivity of polymer towards different cations and affects both the overall physical behavior of sludge and the flocculation capacity. Besides, hydrophobicity is another important property of EPS (Durmaz and Sanin, 2003). Both surface charge and hydrophobicity are important parameters for flocculation of sludges. They are the sum effect of EPS interactions and used as a key to estimate sludge settling efficiency (Boyette et al., 2005).

The surface charge which is carried by colloids generally affects colloid stability. This is important in activated sludge systems, because it will affect the state of flocculation and sedimentation. In activated sludge systems, depending on the composition of bacterial flocs, the sludge may have different surface charges. In general, most activated sludge flocs have a negative surface charge (Garikipati, 2005). Negative surface charge is the result of physico-chemical interactions between microorganisms (mainly bacteria), inorganic particles, EPS and multivalent cations. Shin et al. (2000) state that the ratio of carbohydrates to protein in the EPS is an important factor determining the charge of the cell surface.

Hydrophobicity is a key factor in determining the adhesion potential of microbes to surfaces. Knowledge of cell surface hydrophobicity is important in food processing, environmental engineering, biological system design and other microbial disciplines (Saini, 2010). It is believed that the hydrophobic fraction is made up of proteins. Mostly amino acids contribute to hydrophobicity in EPS structure (Durmaz and Sanin, 2003). Also, hydrophobicity is positively correlated to flocculation. Flocculation is linked to the increased hydrophobicity that includes a mechanism mediated by surface proteins (Xie et al., 2010).

There are some factors that affect surface charge and hydrophobicity such as the composition of feed and the conditions in the aeration tank (Durmaz and Sanin, 2003). Sludge retention time (SRT) also affects these properties. According to Liao et al. (2001), at higher SRTs (16 and 20 days) sludge surfaces are less negatively charged and more hydrophobic than those at lower SRTs (4 and 9 days). It is known that growth and starvation conditions affect some of the bacterial properties such as hydrophobicity, size and biomass and bacterial adhesion. Starved cultures had higher hydrophobicity than growth cultures. Cells starved for short durations (up to 7-10 days) exhibited significant variations in microbial hydrophobicity (Saini, 2010).

1.1. Aim and Scope of the Study

This study is a part of a TÜBİTAK project (Project No. CAYDAG-111Y018, Microbial products and metal inhibition in biological systems) which investigates the inhibitory effect of silver metal on different activated sludges and the relationship between inhibition and EPS characteristics. Figure 1.1. shows the main parts of this project.

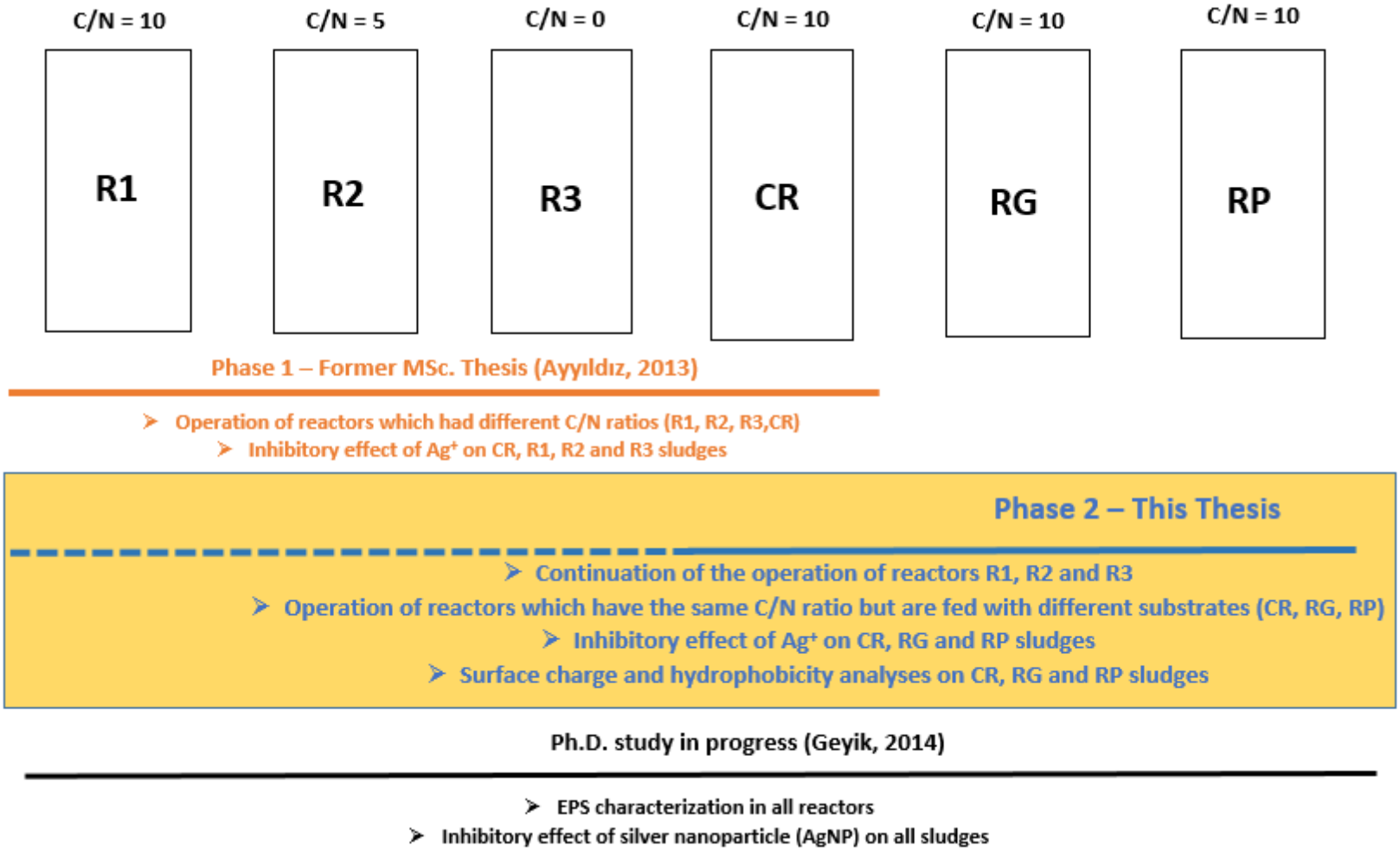

Figure 1.1. The scope of thesis within the TÜBİTAK project.

As shown in Figure 1.1., in the first part of the project, within the scope of a MSc. Thesis, three activated sludge reactors (R1, R2 and R3) were operated at different carbon to nitrogen (COD/TKN) ratios (10, 5 and 0, respectively) and the effect of silver on these sludges were determined (Ayyıldız, 2013). In addition, within the scope of a Ph. D. Thesis, in all activated sludges EPS are characterized as a part of this project (Geyik, 2014). In this Ph. D. study, EPS fractions (Soluble EPS, Loosely bound EPS and Tightly bound EPS) were measured to determine the differences between the activated sludges which are operated at different COD/TKN (C/N) ratios.

This present study also was conducted within the scope of the mentioned project. The aim of the present study was to determine the inhibitory effect of silver on the laboratory-scale activated sludge reactors that were operated at the same carbon to nitrogen (C/N) ratio, but were fed with different organic substrates (namely, a mixture consisting of glucose, peptone and sodium acetate, glucose only and peptone only). Besides, in these sludges the surface charges and hydrophobicities were determined. It was assumed that different feed composition affects EPS production, surface charge and hydrophobicity of activated sludge. As a result, the inhibitory effect of the silver metal on these sludges may change. Also, the speciation of silver might change in different feed solutions.

In this study, three different activated sludges were fed for a long period of time with different synthetic wastewaters that have the same COD/TKN (C/N) ratio. Then, the effect of Ag on these sludge types was determined by respirometry using these sludges. When respirometry tests were conducted, samples were also examined by analytical methods. COD, SS, VSS and pH analyses were conducted in reactors and respirometric tests throughout the study. Moreover, surface charge and hydrophobicity analyses were done on the sludges that were used in respirometric tests.

In addition to the three main reactors, the operation of the reactors that have different COD/TKN ratios and that were started up in a previous work (Ayyıldız, 2013) was also continued. This was necessary since in parallel to the present study, another study was conducted to characterize the EPS of each reactor. The information about the operation of all reactors is presented in **Appendix A.**

2. LITERATURE REVIEW

2.1. Activated Sludge Process

2.1.1. Definition of Activated Sludge Process

The activated sludge process is a continuous or semi-continuous (fill-and-draw) aerobic method for biological wastewater treatment which includes carbonaceous oxidation and nitrification. This process was developed in 1914 and it was named activated sludge because it involved the production of an activated mass of microorganisms capable of aerobically stabilizing a waste.

Activated sludge treatment removes the dissolved and colloidal biodegradable organics from wastewater as well as the unsettleable suspended solids and other constituents which can be sorbed on, or entrapped by, the activated sludge floc. Moreover, the mineral nutrients (phosphorus and nitrogen compounds) can also be partially removed by using this process.

In the activated sludge system, a wastewater, usually domestic wastewater, is stabilized biologically in a reactor under aerobic conditions and the content of the reactor is named as the mixed liquor. The aerobic environment is achieved by using diffused or mechanical aeration. After the treatment of the waste in the reactor, the resulting biological mass is separated from the liquid in a settling tank. A portion of settled biological solids is recycled; the remaining mass is wasted. A portion of the microorganisms must be wasted; if not, the mass of microorganisms would keep increasing until the system could no longer contain them. The level at which the biological mass should be kept depends on the desired treatment efficiency and other considerations related to growth kinetics. Figure 2.1. shows a typical activated sludge process.

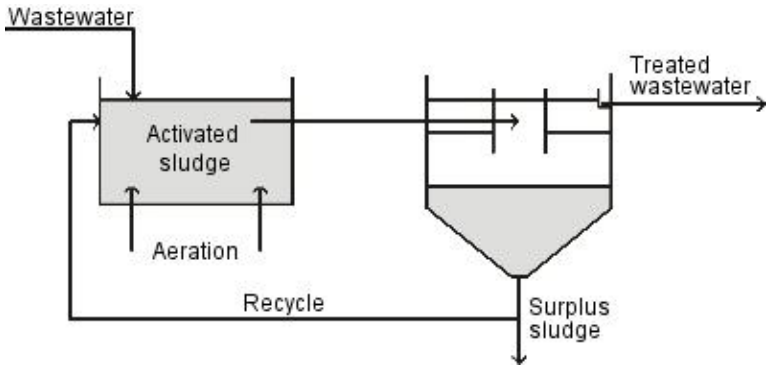

Figure 2.1. Typical activated sludge process (Pombo et al., 2011).

Microorganisms are important in order to design and operate an activated sludge process efficiently. In nature, the key role of the bacteria is to decompose organic matter produced by other living organisms. In the activated sludge process, the bacteria are the most important microorganisms because they are responsible for the decomposition of organic material in the influent. In the mixed-liquor tank, aerobic and facultative bacteria use a portion of the organic waste to obtain energy and the remaining of the organic material to synthesize new cells. Only a portion of the original waste is actually oxidized to low-energy compounds such as NO_3^-, SO_4^{-2} and CO_2; the remaining part is synthesized into cellular material. Also, many intermediate products are formed before the final end products of oxidation are obtained.

In addition, formation of a floc by bacteria is an important issue. A satisfactory floc is a prerequisite for the effective separation of the biological solids in the settling unit. It was observed that as the mean cell residence time is increased, the settling characteristics of the biological floc are improved. The reason is that as the mean age of the cells increases, the surface charge is reduced and the microorganism start to produce extracellular polymers, finally becoming encapsulated in slime layer. The presence of these polymers and the slime promotes the formation of floc particles that can be removed readily by gravity settling (Ganczarczyk, 1983; Metcalf and Eddy, 1972).

2.1.2. Factors Affecting Activated Sludge Process

There are many factors which affect the performance of an activated sludge system, but the most important ones are sludge retention time (SRT), food to microorganism (F/M) ratio, mixed liquor suspended solids (MLSS), dissolved oxygen (DO) and wastewater temperature (Ganczarczyk, 1983).

SRT is defined as the mass of particulates in the bioreactor divided by the mass discharged per unit time. This parameter is the most important design parameter in determining the performance of an activated sludge process. SRT affects many factors in the system, such as nitrification. In addition, this parameter affects floc macrostructure by affecting the relative proportion of floc forming bacteria and filamentous bacteria. The choice of SRT depends on the objective of the plant. High SRT values are chosen for nitrification due to the low specific growth rate of autotrophs. SRT is affected by temperature and substrate complexity. Municipal plants generally have lower SRTs than industrial plants, because of the higher complexity of the substrate in the industrial wastewater (Maharajh, 2010).

The F/M ratio is another important parameter in the activated sludge process. It is also called sludge loading rate or substrate loading rate. This parameter is calculated in grams of BOD or COD per gram of mixed liquor suspended solids (MLSS) or mixed liquor volatile suspended solids (MLVSS) and time. Equation 2.1 shows the calculation of the F/M ratio.

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

where;

Q is the influent wastewater flow rate, m^3/d;

S_0 is the influent BOD or COD concentration, g/m^3;

V is the tank volume, m^3;

X is the mixed liquor biomass concentration (MLSS or MLVSS) in the aeration tank, g/m^3.

Mixed liquor suspended solids are composed of active microbial mass, non-active microbial mass, non-biodegradable organics and inorganic mass. In conventional activated sludge systems treating municipal wastewater, the active microbial mass generally represents only 30% or less of mixed liquor suspended solids. However, in extended aeration activated sludge systems, the active microbial mass is generally less than 10%. The level of MLSS varies widely for different modifications of activated sludge process. Optimization analyses showed that the most suitable and economically attractive range of MLSS is between 2000 – 4000 mg/L.

Lastly, dissolved oxygen concentration and wastewater temperature are the parameters affecting the process. It is said that 1 – 2 mg/L dissolved oxygen in mixed liquor is sufficient for activated sludge treatment. The requirement for the minimum level of dissolved oxygen in aeration tanks depends on the mixing characteristics and the level of MLSS. Temperature affects wastewater viscosity and surface tension. Moreover, the temperature affects the rates of biological reactions in the reactor. For this reason, optimum temperature should be provided for the system (Ganczarczyk, 1983).

2.1.3. Substrate Utilization in Activated Sludge

Microorganisms must have carbon sources for synthesis of new cellular material, sources of energy and inorganic elements (nutrients) such as nitrogen, phosphorus, calcium and magnesium. Bacterial populations are grouped with respect to cell carbon source and energy production. Organic matter and carbon dioxide are the carbon sources for cell growth. Heterotrophic organisms use organic carbon for cell synthesis and autotrophs use carbon dioxide to form new cells (Tchobanoglous et al., 2003). In Figure 2.2. some examples of bacterial metabolism are given.

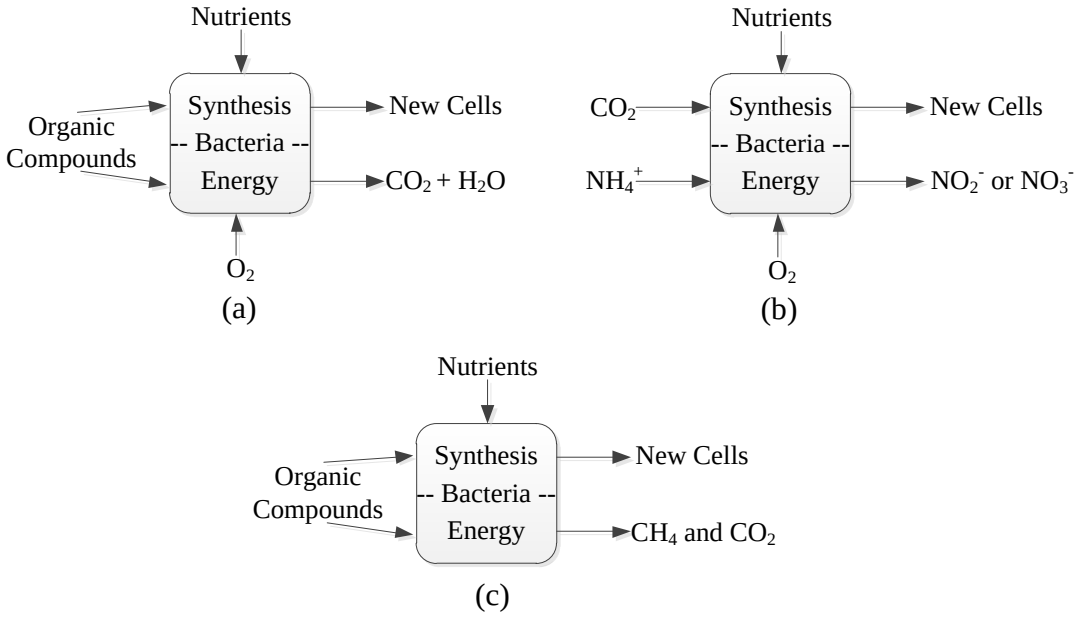

Figure 2.2. Examples of bacterial metabolisms: (a) aerobic, heterotrophic, (b) aerobic, autotrophic, (c) anaerobic, heterotrophic (Tchobanoglous et al., 2003).

The aerobic systems for treating organic wastewater depend upon the physiology of heterotrophic organisms. In the presence of oxygen, these organisms use the organic substances present in the wastewater both as a carbon source for cell synthesis and as an energy source. Theoretically, when wastewater contacts with the microorganism in the presence of dissolved oxygen, the suspended and colloidal solids in the wastewater are adsorbed on the surface of activated sludge flocs. On the other hand, intensive biological activity converts some part of the wastewater organics into a reserve food inside microbial cells. As a result, these two processes combined are responsible for the initial organic substrate removal in activated sludge system (Ros, 1993).

2.1.3.1. Organic Carbon Removal: The major application of biochemical operations to wastewater is the removal of soluble organic matter. This process occurs as the microorganisms use it as a food source. A portion of the carbon in the organic matter is converted into new biomass and the remainder is converted into carbon dioxide (Grady et al., 2011).

The organic content of a wastewater is generally measured by two parameters which are Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). COD is a useful parameter for the modelling of biological kinetics, because it sets electron equivalence of the substrate, biomass and oxygen requirement. Also, it reflects biodegradable organics and residual components of wastewater (Orhon et al., 1997).

Total COD in wastewater can be divided into two groups: total nonbiodegradable (inert) COD and total biodegradable COD. Total inert COD can be subdivided into soluble inert COD and particulate inert COD. Also, the biodegradable COD can be divided into the readily biodegradable COD and slowly biodegradable COD. The characteristic feature of the readily biodegradable COD is that it can be directly absorbed for synthesis. On the other hand, hydrolysis is required first for the utilization of the slowly biodegradable COD. So, slowly biodegradable COD is grouped as rapidly and slowly hydrolysable COD. The fractionation of total COD is given in Figure 2.3 (Orhon and Çokgör, 1997; Orhon et al., 1997).

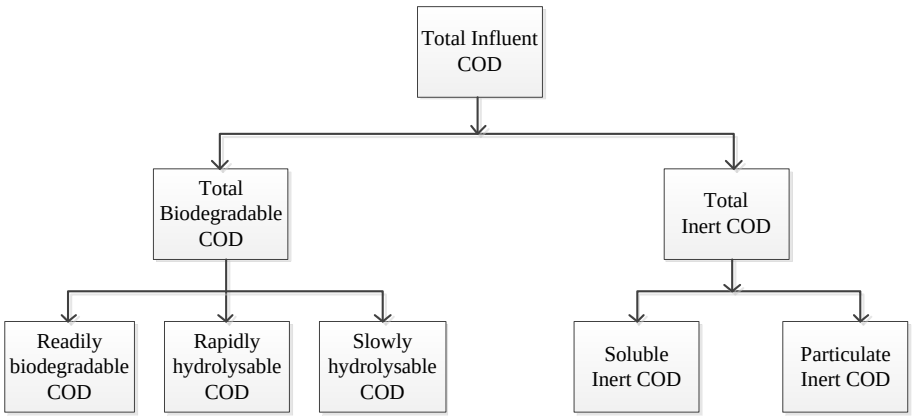

Figure 2.3. COD fractionation in a wastewater (Orhon and Çokgör, 1997).

The substrate utilization can be examined on the basis of electron transport. Under aerobic conditions, when microorganisms use an electron-donor substrate for synthesis, a portion of its electrons (f^0_e) is initially transferred to the electron acceptor (oxygen) to provide energy. The other portion of electrons (f^0_s) is converted into microbial cells as given in Figure 2.4. The sum of f^0_e and f^0_s is 1 which represents the total amount of substrate

(Rittmann and McCarty, 2001). Oxygen consumption values obtained from respirometric tests represent the part of the substrate which is used in the energy reaction.

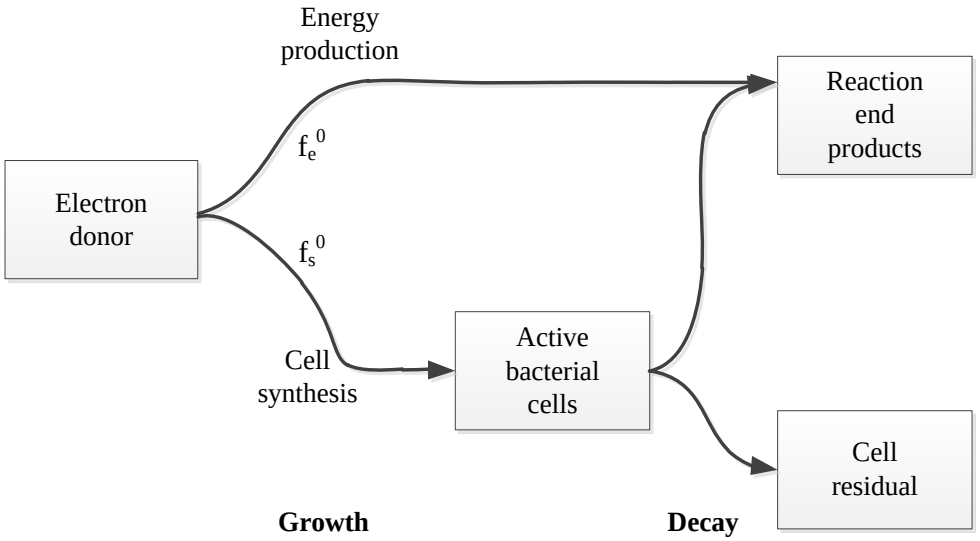

Figure 2.4. Utilization of substrate for energy production and synthesis (Rittmann and McCarty, 2001).

The fraction f^0_s can be converted into mass units: g cell COD produced/g COD consumed. If it is expressed in mass units, it is termed the true yield coefficient which is represented as Y as shown in Equation 2.2.

$$Y = f^0_s \text{ (MW g cells/mol cells)}/[(n_e \text{ e}^- \text{ eq/mol cells})/(8 \text{ g COD/ e}^- \text{ eq donor})] \quad (2.2)$$

where MW is molecular weight of cells; n_e is number of electron an equivalents in an empirical mole of cells.

The growth rate of microbial cells is expressed as in Equation 2.3.

$$dX_a/dt = Y\left(\frac{-dS}{dt}\right) - bX_a \quad (2.3)$$

where dX_a/dt shows the net growth rate (M/L^3T) of active microorganism (X_a, M/L^3), $-dS/dt$ shows the rate of consumption (M/L^3T) of substrate (S, M/L^3), 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:

$$\mu_{syn} = \left(\frac{1}{X_a} \frac{dX_a}{dt} \right)_{syn} = \hat{\mu} \frac{S}{K+S} \tag{2.4}$$

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

In the absence of substrate, the cells oxidize themselves to meet maintenance-energy needs. This is termed as endogenous respiration and represented as μ_{decay}. It is shown in Equation 2.5. b symbolizes the endogenous decay coefficient (1/T).

$$\mu_{decay} = \left(\frac{1}{X_a} \frac{dX_a}{dt} \right)_{decay} = - b \tag{2.5}$$

Substrate utilization is mathematically represented in the following equation.

$$r_{ut} = - \frac{\hat{q} S}{K+S} X_a \tag{2.6}$$

where r_{ut} represents the rate of substrate utilization (M/L^3T) and $\hat{q}$ is the maximum specific rate of substrate utilization (M/MT). Substrate utilization and biomass growth are connected by the following equation:

$$\hat{\mu} = \hat{q} Y \tag{2.7}$$

in which Y is true yield for biomass synthesis. It represents the fraction of electron-donor electrons converted to biomass electrons during the new biomass synthesis. So, the net rate of cell growth can be represented as:

$$r_{net} = - Y \frac{\hat{q}\, S}{K+S} X_a - b X_a \qquad (2.8)$$

$$\mu = r_{net} / X_a = - Y \frac{\hat{q}\, S}{K+S} - b \qquad (2.9)$$

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/g substrate COD. Moreover, for heterotrophic microorganisms $\hat{\mu}$ is between 8.4 and 13.2 d^{-1} and $\hat{q}$ is in the range of 20-27 g COD/g VSS.d (Rittmann and McCarty, 2001).

2.1.3.2. Nitrification: Nitrification is the microbiological oxidation of ammonia nitrogen (NH_4^+-N) to nitrite nitrogen (NO_2^--N) and nitrate nitrogen (NO_3^--N). Nitrifiers represent the microorganisms responsible for this oxidation. The nitrifying bacteria are autotrophs, chemolithotrops and obligate aerobes. Autotrophs can fix and reduce inorganic carbon. This requires much energy which is primarily responsible for nitrifiers having much smaller values of f^0_s and Y than heterotrophs. The chemolithotrophic character makes f^0_s and Y smaller because nitrogen electron donors of nitrifiers give less energy per electron equivalent than organic electron donors. The low Y value causes a small maximum specific growth rate. As a result, nitrifiers are slow growers. They can be inhibited more than heterotrophs by a large variety of toxicants. Lastly, nitrifiers use O_2 for respiration and as a direct reactant for the initial monooxygenation of NH_4^+ to NH_2OH (hydroxylamine). Moreover, nitrifiers are intolerant of low oxygen concentration (Rittmann and McCarty, 2001; Madoni et al., 1999).

Nitrification is a two-step process. In the first step, NH_4^+-N is oxidized to NO_2^--N according to following reaction:

$$1/6\ NH_4^+ + 1/4\ O_2 \rightarrow 1/6\ NO_2^- + 1/3\ H^+ + 1/6\ H_2O \qquad (2.10)$$

The second step is the oxidation of NO_2^- to NO_3^- according to following reaction:

$$1/2\ NO_2^- + 1/4\ O_2 \rightarrow 1/2\ NO_3^- \quad (2.11)$$

The total nitrification reaction can be expressed as follows:

$$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + 2H^+ + H_2O \quad (2.12)$$

The f^0_s and Y value of ammonium oxidizing bacteria (AOB) are 0.14 and 0.33 mg VSS/ mg NH_4^+-N, respectively. For nitrite oxidizing bacteria (NOB), these values are 0.10 and 0.083 mg VSS/ mg NO_2^--N, respectively. $\hat{\mu}_n$ of the AOB and NOB is in the range of 0.32 - 1.02 d^{-1} and 0.34 – 1.1 d^{-1} with respect to temperature (Rittmann and McCarty, 2001).

2.1.4. Extracellular Polymeric Substances of Activated Sludge

Bacterial extracellular polymeric substances (EPS) are the major exopolymers on bacterial surface. EPS are defined as any polysaccharide or peptidoglycan structure of bacterial origin which is lying outside the cell membrane. They are responsible for increasing the bridging in flocculation and help the formation of a well-settling floc. In addition, they help retain floc structures and minimize shear effects (Shin et al., 2000; Boyette et al., 2005).

There are some functions of EPS which are adhesion to surfaces, aggregation of bacterial cells in flocs and biofilms, stabilization of the biofilm structure, formation of a protective barrier to provide resistance to biocides or other harmful effects, sorption of exogenous organic compounds for the accumulation of nutrients from the environment, and accumulation of enzymatic activities, such as digestion of exogenous macromolecules for nutrient acquisition. Moreover, EPS allow microorganisms to live continuously at high cell densities in stable mixed population communities (Laspidou, 2003).

EPS are metabolic products which contain various organic substances such as exopolysaccharides (PS), exoproteins (PN), DNA, humic acids, uronic acids and so on. As mentioned before, they form a buffering layer for the cell against the harsh external environment, and also they serve as a carbon and energy source during starvation (Wang et

al., 2006). EPS are originated from metabolism of microorganisms and the wastewater itself, and are made up of loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) (Ye et al., 2011). In the past, research assumed that polysaccharide is the most abundant component of EPS. However, proteins and nucleic acids also appear in significant amounts or even predominate in EPS from several sources (Laspidou, 2003). According to some studies, protein was the principal component, and carbohydrate was the second component of the EPS matrix in the activated sludge system. Therefore, protein and carbohydrate of EPS are generally analyzed in the studies (Ye et al., 2011).

The nature and content of EPS are sensitive to environmental and operational conditions. If culture conditions change, the nature of polymers changes. Nutritional parameters, for example the carbon to nitrogen (C/N) ratio, affect the microbial physiology, so affecting also the nature and content of EPS (Ye et al., 2011).

Most EPS are anionic and nonionic in nature and this property determines the surface charge and selectivity of polymer towards different cations and affects both the overall physical behavior of sludge and the flocculation capacity. Besides, hydrophobicity is another important property of EPS (Durmaz and Sanin, 2003). EPS structure contributes a huge surface area. EPS are the key components that determine the physicochemical and biological properties of sludge, such as surface property, settlement, dewaterability, etc. EPS contain anionic functional groups, such as carboxylic and phosphate. The ionization of this kind of groups makes EPS carry negative charges. This surface charge property of EPS causes hydrophobic and ionic interactions and hydrogen bonding which are responsible for the affinity of EPS. The negatively charged EPS combine flocs together when it is bridged by cations (Tian et al., 2006).

Hydrophobicity is another important characteristic of EPS. Hydrophobic effect results from the behavior of particles incapable of interacting electrostatically or establishing hydrogen bonds with water. EPS contain many hydrophobic groups, but proteins and carbohydrates have a great influence on hydrophobicity. Wrangstadh et al. (1986) stated that cells in starvation had a lower hydrophobicity because of the release of hydrophilic EPS carbohydrates; and the hydrophobicity increased 10 h later when the EPS carbohydrates were consumed by endogenous respiration. Moreover, in their study Jorand et al. (1998)

found that there were not any carbohydrates in the hydrophobic fraction of activated sludge. These results suggest that proteins in EPS contribute to the hydrophobicity of biomass, and not carbohydrates (Tian et al., 2006).

EPS are also effective in binding heavy metals. Two types of mechanisms may be involved: (1) ion exchange due to high amount of negatively charged functional groups like sulfate, carboxyl and phosphate groups in EPS; (2) complexation with negatively charged functional groups. The electrostatic interactions lead to formation of stable complexes and EPS bind some metals to the cell surface (Tian et al., 2006; Pal and Paul, 2008). This point is particularly important within the scope of this thesis.

2.1.5. Surface Charge of Activated Sludge

2.1.5.1. Definition and Properties: Surface charge is an important parameter to determine the flocculation of activated sludge. The surface charge which is carried by colloids generally affects colloid stability. In activated sludge systems this is important, because it will affect the state of flocculation and sedimentation. Activated sludges, depending on the composition of bacterial flocs, may have different surface charges. In general, most activated sludge flocs have a negative surface charge (Garikipati, 2005). Negative surface charge is the result of physico-chemical interactions between microorganisms (mainly bacteria), inorganic particles, EPS and multivalent cations. Strong negative charge is more favorable for bacterial aggregation, because bacterial surfaces and EPS provide negatively charged adsorption sites for divalent cations such as Mg^{2+} and Ca^{2+}. Divalent cations may act as bridging agents between the extracellular organic constituents of the flocs (Shin et al., 2000). Additionally, it is believed that the charge is related to the amount of extracellular polymeric substances (EPS) on the surface; changes in bacterial growth conditions will affect the content and composition of EPS and thereby the surface charge (Mikkelsen, 2003).

Microorganisms obtain a surface charge through ionization of carboxyl and amino groups which are negatively charged at high pH, positively charged at low pH and neutrally charged at the isoelectric point. When a particle is charged, ions of the opposite charge are attached to the surface. The potential at the surface of this cloud of counter ions is called the

zeta potential. This zeta potential of solids in suspension is measured in terms of the electrophoretic mobility of solids (Wilen, 1995).

The DLVO theory (Derjaguin, Landau, Verwey and Overbeek theory) is a model which describes the balance of forces between charged colloid particles. This theory is also named as double layer theory because it describes charged particles as having a double layer of counter ions surrounding the particle. The first layer is referred as the Stern layer which is comprised of a tightly associated layer of counterions, and the second layer is often referred to as the diffuse layer which is made up of less tightly associated counterions. When the distance from the particle surface increases, the concentration of ions in the diffuse layer decreases until the concentration of ions equals that of the bulk solution. The result is an electric potential that develops around the particle (Adamson, 1990; Vatansever, 2005). When two similar colloidal particles with similar primary charge approach each other, their diffuse layers begin to interact. The similar primary charges they possess result in repulsive forces. Repulsive forces which keep particles from aggregating are counteracted to some degree by an attractive force termed van der Waals attraction. All colloidal particles possess this attractive force regardless of charge and composition. As particles with similar charge approach one another, the repulsive electrostatic forces increase to keep them separated. However, if they can be brought sufficiently close together to get pass this energy barrier, the attractive van der Waals force will predominate, and the particles will remain together. The random motion of colloids caused by the constant collisions with water molecules, termed Brownian movement, will bring particles in close proximity and aggregation may occur. However, coagulants and polymers are typically added to lower the energy barriers between particles and provide efficient agglomerations for settling (Garikipati, 2005). Particle surface charge distribution is shown in Figure 2.5.

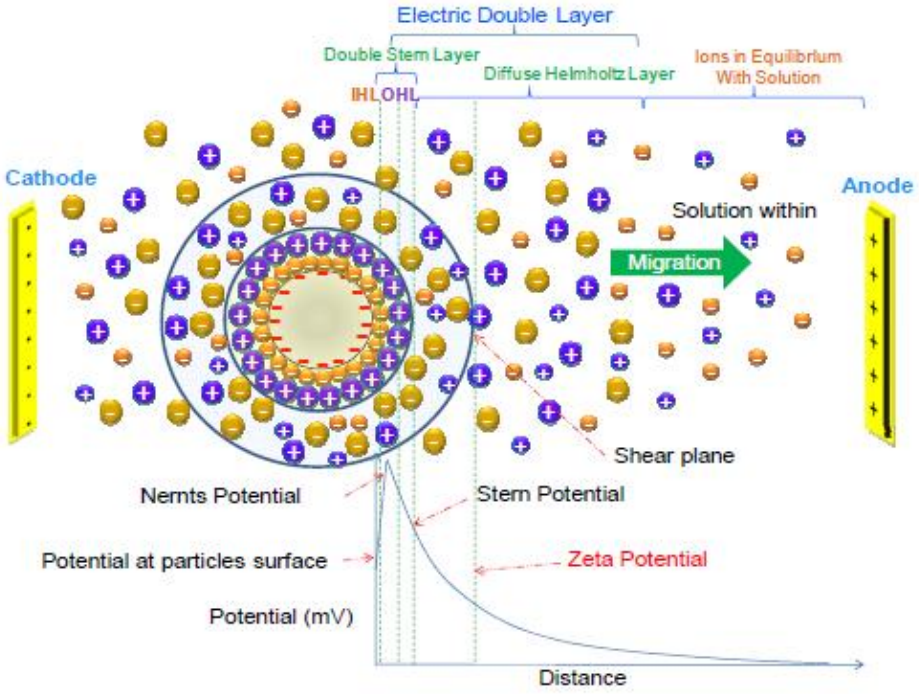

Figure 2.5. Particle surface charge distribution (Maldonado et al., 2012).

It is believed that protein to carbohydrate ratio (P/C ratio) in determining the surface charge is related to the unique charge properties of proteins. The amino groups in proteins carry positive charges and can neutralize some of the negative charge from carboxyl and phosphate groups. As a result, the net negative surface charge of flocs decreases (Liao et al., 2001). Additionally, composition of wastewater may affect the surface charge of activated sludge. Recent research indicates that the C/N ratio of the feed determines the overall composition of EPS, thus the C/N ratio possibly affects the physical properties of sludge (Durmaz and Sanin, 2003). Shin et al. (2000) state that the ratio of carbohydrates to protein in the EPS is an important factor determining the charge of cell surface. According to their study, the negative surface charge was decreased as the ratio of carbohydrate to protein in EPS increased. The study showed that EPS composition affects the surface charge of cells. Moreover, an increase in the ratio of carbohydrates to proteins inhibited floc formation by increasing the cell surface charge.

In their study, Durmaz and Sanin (2003) used different wastewaters which had different C/N ratios. They observed a higher surface charge with the increase of the C/N ratio. The changes in these surface properties were related to the distribution of proteins and

polysaccharides in the EPS, such that with the production of higher quantities of carbohydrates, the electronegativity increased. Additionally, SRT also affects the surface charge of activated sludge. In a research Liao et al. (2001) studied with wastewaters which had different SRT values ranging from 4 to 20 days. According to their results, similar surface charges were observed for sludges operated at 4 and 9 day SRT or 16 and 20 day SRT. Moreover, at higher SRTs, smaller surface charges were observed compared to lower SRTs.

2.1.5.2. Methods for Measuring Surface Charge: There are different methods to determine surface charge. These are zeta potential, pH titration and colloidal titration.

Zeta potential measurements are based on a small number of particles and may not generally be representative of the suspension because some large particles may settle during measurements. The zeta potential quantifies the potential at the plane of shear, which is not identical to the surface potential. The biggest problem in adopting zeta potentials in charge quantification is, however, that the zeta potential quantifies the density of particle charges irrespective of particle size and numbers. The zeta potential thus may not be related to the total surface charge of the suspension, when the particle number varies (Mikkelsen, 2003). According to a study of Mikkelsen and Keiding (2002), the zeta potential of activated sludge is in the range of -29.6±8.5 mV. In addition, Chao and Keinath (1979) observed that the average zeta potential was -24.9 mV (Vatansever, 2005).

In the pH titration method, titration is not limited to surface. pH titration gives a measure of the total amount of ionisable surface groups in a suspension, for a typical acid titration, weak acid groups (which contribute to surface charge) and weak base groups (which do not contribute to surface charge). In some studies, it is stated that in pH titration a higher charge was observed (Mikkelsen, 2003).

The colloidal titration method is the best method to be used in activated sludge studies, not only to measure the surface charge of flocs, but also to measure the surface charge of EPS. This procedure was developed for determination of polymer charges in dilute solutions, but may be used for characterization of organic and inorganic particles (Mikkelsen, 2003). This method is based on a stoichiometric reaction between surface charges and standard

polymer reactants. Excess of cationic polymer reactant is titrated with an anionic polymer reactant and the actual net surface charge of a suspension containing both negative and positive surface groups can be estimated (Garikipati, 2005).

Colloid titration is based on the reaction between positively and negatively charged polyelectrolytes. The polyelectrolytes are kept stable in aqueous solutions by their charges. If their charges are neutralized by the polyelectrolytes of the opposite charge, the polyelectrolytes tend to associate and eventually precipitate. Accordingly, when an aqueous solution containing a positive polyelectrolyte is added to an aqueous solution containing a negatively charged one, the neutralization reaction will proceed stoichiometrically. The reaction is shown in Figure 2.6.

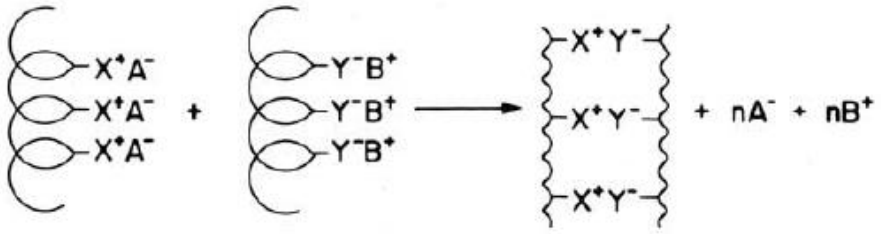

Figure 2.6. Reaction between positive and negative polyelectrolytes (Ueno and Kina, 1985).

If the selected titrant is a positive polyelectrolyte and its chemical structure, molecular weight or equivalent weight are known, the negative polyelectrolyte in the sample solution can be determined volumetrically. In general, the procedure is similar to neutralization titration and the end point is easily detected with the use of suitable visual indicators.

The recommended polyelectrolytes for colloid titration were glycolchitosan (Gch) and potassium poly(vinyl)sulfate (PVSK) as positive and negative polyelectrolytes, respectively. The reaction is stoichiometric, so that the aqueous solution of Gch can be titrated with an aqueous solution of PVSK of known concentration. Toluidine Blue which is a cationic blue colored dye is recommended as a visual indicator in this titration. This indicator does not bind with positively charged Gch, but it binds with PVSK and becomes red-violet. This color change is so sensitive that a sharp endpoint can be expected. In the titration of Gch with PVSK, the solution becomes turbid, but it stays blue before the endpoint because the reaction between Gch and PVSK predominates over the reaction between Toluidine Blue and PVSK.

After the endpoint, a minute excess of PVSK binds with Toluidine Blue, resulting in the color change from blue to red-violet. Near the endpoint, the turbid precipitates aggregate and the supernatant solution becomes clear, so that the endpoint color change can easily be detected (Ueno and Kina, 1985).

There are many studies about surface charge measurements in activated sludges. In all studies, different positive polyelectrolytes have been used. In a study, Cat-Floc was used and results showed that the surface charge was around -0.25 to -0.50 meq/g MLSS. In another study, Bura et al. (1998) used polybrene as a positive polyelectrolyte and the surface charge of activated sludge was found between -0.25 to -0.54 meq/g MLSS (Mikkelsen, 2003).

2.1.6. Hydrophobicity of Activated Sludge

2.1.6.1. Definition and Properties: As mentioned before, hydrophobicity is a key factor in determining the adhesion potential of microbes to surfaces. Knowledge of cell surface hydrophobicity is important in many areas including environmental engineering (Saini, 2010). It is believed that cell surface hydrophobicity is important for flocculation and sludge settling. Higher hydrophobicity produces higher adhesion to flocs and is positively correlated to flocculation (Xie et al., 2010).

Hydrophobic fraction is made up of proteins and mainly amino acids contribute to hydrophobicity in EPS structure. Glycine, alanine and leucine are the most important amino acids in extracellular proteins that have hydrophobic properties, as a result they are likely to be involved in hydrophobic interactions (Durmaz and Sanin, 2003).

On the other hand, it was observed that the total carbohydrate levels had a negative influence on hydrophobicity. It was concluded that the presence of a large amount of hydrophilic and mainly neutral carbohydrates may be contributing to the more hydrophilic nature of sludge (Liao et al., 2001; Durmaz and Sanin, 2003). Additionally, the C/N ratio also affects the hydrophobicity of activated sludge. In their study, Durmaz and Sanin (2003) showed that hydrophobicity of the sludge decreases with the increase in the C/N ratio. At high C/N ratios, the system becomes carbon limited and the amount of nitrogen in the feed is higher than required nitrogen by the microorganisms. Microorganisms utilize this excess

nitrogen in the synthesis of proteins. Increasing C/N ratios cause a decrease in the EPS protein/carbohydrate ratio and this means that as the C/N ratio decreases, the sludge EPS contains much higher proteins than carbohydrates. As a result, higher hydrophobicity is observed with decreasing C/N ratio and this indicates the hydrophobic fractions on the cell surface are made up of proteins but not carbohydrates.

According to Shin et al. (2000), surface charges play a role in the hydrophobicity of bacteria. Cells with a largely negative surface charge will be more hydrophobic and they can easily associate to positively charged inorganic particles, for example Mg^{2+} and Ca^{2+}. On the other hand, in their study Liao et al. (2001) state that there is a strong inverse correlation between hydrophobicity and surface charge of sludge. This can be explained by the fact that surface charge is related to the ionizable groups on the sludge surfaces and it increases the polar interactions of EPS with water molecules. As a result, the more charged sludge surfaces become the lower is their hydrophobicity.

2.1.6.2. Methods for Measuring Hydrophobicity: Hydrophobicity is believed to affect mobility, aggregation and attachment characteristics of sludge. There are different laboratory assays for measuring bacterial hydrophobicity. Examples are contact angle measurement (CAM), microbial adhesion to hydrocarbons (MATH), salt aggregation test (SAT) and hydrophobic interaction chromatography (HIC). In all these techniques, MATH is a simple and quick method to measure hydrophobicity. This technique has been used widely in broad areas of environmental engineering (wastewater treatment, biofiltration, and bioremediation), medicinal sciences, food and dairy industry (food and poultry infection) and microbial transport.

MATH test is based on differential partitioning of microbes at a hydrocarbon-aqueous interface. Briefly, in this method the visible absorbance of the aqueous phase before and after vortex mixing a microbial suspension with a hydrocarbon in a certain volume ratio and allowing for phase separation is measured. The difference in absorbance is used as the measure of number of microbes that have partitioned into hydrocarbon phase. The result is reported as cell surface hydrophobicity and mostly presented as the percentage of cells that partitioned into the hydrocarbon phase.

There are studies about the effects of operating parameters of the MATH test. These parameters are hydrocarbon selection, hydrocarbon-aqueous phase volume ratio and vortex duration. Commonly used values of these parameters range between 0 to 5 min vortex duration, 5 to 45 min of phase separation, 0.01 to 1 volume ratio of hydrocarbon to aqueous phase, 400 to 660 nm absorbance wavelength and different hydrocarbons (benzene, toluene, dodecane, octane, etc.). In the research, Saini (2010) aimed to determine the differences in hydrophobicity results when in MATH test operating parameters differed. Different vortex durations, phase separation periods, hydrocarbon to aqueous phase volume ratios, absorbance wavelength and hydrocarbons were selected during the tests. According to the results, for MATH test the following parameters were suggested: a vortex duration of 2 minutes, dodecane as the preferred hydrocarbon using a hydrocarbon-aqueous volume ratio of 1 mL to 4 mL, wavelength of 600 nm. For the separation period 15 to 30 minutes were suggested.

2.2. Inhibition of Activated Sludge by Silver

2.2.1. Properties and Sources of Silver

Silver (Ag) is a transition metal with the atomic number of 47 and the atomic weight of 107.87 g/mol. The toxicity of silver ranges depending on the silver species and the medium in which silver is found. Silver is found in the environment in four oxidation states: 0, 1^+, 2^+ and 3^+. Ag^0 and Ag^+ are the most widely seen forms; Ag^{2+} and Ag^{3+} are rarely found in nature (Purcell and Peters, 1998). Silver is a white, ductile metal occurring naturally in pure form and in ores. Some silver compounds are extremely photosensitive and are stable in air and water, except for tarnishing readily when exposed to sulfur compounds. Although metallic silver is insoluble in water, many silver salts, for example silver nitrate ($AgNO_3$), are soluble. In the natural environment, silver occurs primarily in the form of the sulfide (Ag_2S) or is associated with other metal sulfides, especially those of lead, copper, iron, and gold. Silver readily forms complexes with antimony, arsenic, selenium, and tellurium (WHO, 2002).

Silver is also widely distributed in natural waters throughout the world. It is commonly associated with mineral belts. Different forms of silver can be found in natural waters near many metal mining and milling operations (Rodgers et al., n.d.)

Various forms of silver are used in commerce, and silver is widely transported. Silver metal is used in jewelry and silverware, for alloys and electroplating, and in the processing of food and beverages. Also, silver nitrate is used in the photographic industry, ink manufacturing, coloring porcelain, and as an antiseptic. Traces of silver from these sources can reasonably be expected to reach receiving waters and could be potentially harmful to aquatic biota (Rodgers et al., n.d.). Photoproccessing facilities produce wastewaters having 1.1 and 0.4 mg/L Ag concentrations depending on the absence or presence or recovery, respectively. In one industrial wastewater sample from the photographic film industry, the Ag concentration was 0.077 mg/L. It was reported that the total silver concentration in publicly owned treatment works (POTWs) ranged from 0.004 to 0.10 mg/L (Çeçen et al., 2010). In industrial effluents higher concentrations can be seen.

Silver flow from industrial applications to the environment is shown in Figure 2.7. This figure shows that silver releases into the environment are mostly in the form of solid wastes such as electronic wastes, photographic wastes and batteries. Additionally, silver initially present in a wastewater is wasted with sewage sludge from wastewater treatment plants (Purcell and Peters, 1998).

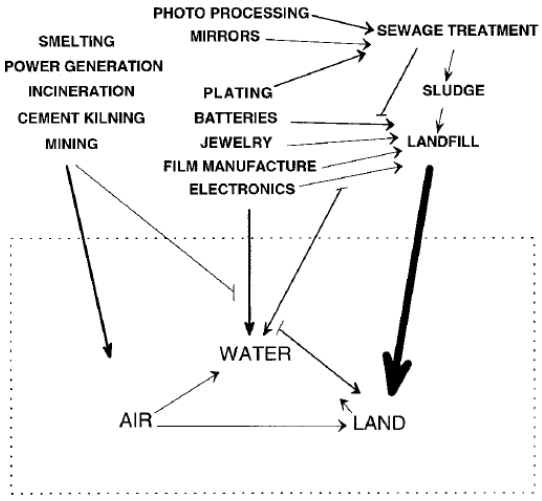

Figure 2.7. Silver flow from industrial applications to the environment (Purcell and Peters, 1998).

2.2.2. Inhibitory Effect of Silver

Heavy metals are toxic to most microorganisms at specific concentrations and often cause serious problems in biological wastewater treatment plants. Heavy metals block the enzyme systems or interfere with some essential cellular metabolite of bacteria and protozoa. The toxicity of heavy metals in activated sludge mainly depends on two factors which are metal species and concentration. Also, other factors such as pH, sludge concentration, influent strength are reported to affect the toxicity of metals. It is usually reported that only soluble metal ions are toxic to activated sludge (Sa'idi, 2010).

Studies mention that heavy metals can change the microbial structure of activated sludge by modifying both cell density and species richness, even at moderate concentrations. Heavy metals affect the metabolic functions of microorganisms in activated sludge and decrease the effectiveness of the biological processes in wastewater treatment plants (Hartmann et al., 2013). There are many studies which indicate the inhibitory effects of heavy metals on activated sludge systems. In their study Ong et al. (2010) stated that increasing heavy metal concentrations resulted in oxygen uptake rate reduction in activated sludge. It was observed that when heavy metals were added, biodegradation activities of microorganisms were inhibited.

Silver is one of the most toxic heavy metals. The bacteriostatic effect of silver was recognized as early as in later parts of 19^{th} century when the use of silver in water treatment was practiced. It is believed that silver toxicity is associated with the free silver ion and is not a function of the concentration of total silver.

It was reported that an experimental activated sludge system was able to treat silver-bearing photoprocessing wastewaters with a total silver concentration in the mixed liquor over 150 mg/L with no adverse effects. However, the silver was present as silver sulfide (Ag_2S) with some metallic silver. As a result of the study, both silver species were removed by sludge settling leading to a very high silver removal efficiency (>90%). It was observed that there was no toxicity to unacclimated activated sludge microorganisms by silver thiosulfate at silver levels of 100 mg/L, but 6.4 mg Ag/L added in the form of silver nitrate

($AgNO_3$) resulted in about 84% inhibition in respirometric studies (Pavlostathis and Maeng, 1998).

In another study, Çeçen et al. (2010) tested the effects of Cd, Pb, Hg, Ag and two different forms of Cr (Cr^{3+} and Cr^{6+}) on nitrifying activated sludge by using a respirometric method. Results of the study showed that Ag was the most toxic metal among all these metals. On molar basis the toxic effect of this metal was one to two orders magnitude higher than other metals. It was observed that Ag at very low concentrations had a high inhibitory effect on sludge. The Ag concentration leading to 10% inhibition was about 0.07 mg/L and 90% inhibition was observed at 1.01 mg/L. As a result of the study, it was concluded that the free form of Ag is directly taken onto or into biomass causing toxicity.

2.3. Sorption of Heavy Metals on Activated Sludge

Biological sludge has a high tendency for various metals. Biosorption of heavy metals can employ different biomasses and different mechanisms for example, chelation, ion exchange and adsorption by physical forces. The concentration range, existence of other metals and the speciation of metals are important factors (Çeçen and Gürsoy, 2001).

It is stated that metals interact with EPS and may act as bridging mechanisms within the negatively charged matrix of EPS. There are some processes that affect the uptake of metal by microorganisms. Some of them are interactions with the EPS, intracellular uptake through the cell surface, association with cell surface, interaction with cellular metabolites and through transformation and subsequent volatilization of the metal. Anionic and neutral polysaccharides in the EPS have different metal adsorption sites and metal ions of different valencies bind differently with the floc. This shows that different metals have different influences on floc properties (Finlayson, 1998).

The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ions). Sorbate species are attracted and removed by different mechanisms due to higher affinity of the sorbent for the sorbate. This process continues until equilibrium is established between the amount of solid-bound sorbate species and its portion

remaining in the solution. The degree of sorbent affinity for the sorbate determines its distribution between the liquid and solid phases. The major factors that affect the biosorption processes are temperature, pH, initial metal concentration and biomass concentration in solution. In all these, pH is the most important parameter. It affects the solution chemistry of the metals, the activity of the functional groups in the biomass and the competition of the metallic ions (Das et al., 2008).

The Langmuir and the Freundlich adsorption isotherms are generally used to evaluate the adsorption and biosorption data. These isotherm equations are used to describe the equilibrium state for single-ion adsorption experiments. The theoretical basis of the Langmuir equation relies on the assumption that there is a finite number of binding sites which are homogeneously distributed over the adsorbent surface of the absorbent, having the same affinity for adsorption of a single molecular layer, and there is no interaction between adsorbed molecules. Langmuir isotherm is shown in the following equation:

$$q = q_m \times b \times C_e / (1 + b \times C_e) \quad (2.13)$$

where, q is the amount of metal adsorbed, mg/g; q_m is the maximum metal uptake value corresponding to sites saturation, mg/g; C_e is the equilibrium metal concentration in solution, mg/L; and b is the ratio of adsorption/desorption rates, representing the biomass–metal binding affinity.

The Freundlich equation is an empirical relationship and it is assumed that the adsorption energy of a metal binding to a site on an adsorbent depends on whether or not the adjacent sites are already occupied. Freundlich equation is shown in the following equation:

$$q = k \times C_e^{1/n} \quad (2.14)$$

where k and n are constants indicating adsorption capacity and adsorption intensity, respectively (Lei et al., 2008).

As mentioned before, these models can be applied at a constant pH and used for modelling of biosorption equilibrium in the presence of a single metal (Das et al., 2008).

2.4. Respirometry of Activated Sludge

Respirometry is the measurement of respiration rate of activated sludge, and it is defined as the amount of oxygen per unit volume and time that is consumed by microorganisms (Gernaey et al., 2001). Oxygen consumption is directly connected with both substrate removal and biomass growth, so respirometry is a useful technique for monitoring, modelling and control of the activated sludge process. In the past, Biochemical Oxygen Demand (BOD) of wastewater was the main focus, but nowadays, respirometry is an instrumental alternative to the original BOD-test which depends on chemical analysis of oxygen concentration.

Respiration rate is measured by using respirometers. All respirometers are based on a technique for measuring the rate at which biomass takes up dissolved oxygen (DO) from the liquid. This can be done directly by measuring DO or indirectly by measuring gaseous oxygen (Vanrolleghem, 2002).

In the past, many respirometric principles were developed, but these can be classified into a number of basic measurement principles which depend on two criteria: (1) The phase where oxygen is measured (liquid or gas), and (2) The flow regime of both liquid and gas phase that can be either static or flowing. The flowing gas-static liquid respirometers are continuously aerated and higher sludge concentrations can be used. This is regarded as an advantage of these respirometers. In these systems, there is a continuous input of oxygen to avoid oxygen limitation (Gernaey et al., 2000).

Respirometers measure the decrease in DO concentration with respect to time by using a DO sensor. The relationship between the time and the decrease is normally linear and the oxygen uptake rate can be calculated from the slope of the curve. Oxygen utilization rate (OUR) is reported as mg O_2/L.min or mg O_2/L.h. If the OUR value is related to MLVSS concentration, the specific oxygen uptake rate (SOUR) can be obtained. SOUR value is represented the amount of oxygen used by a known amount of microorganisms and reported as mg O_2/mg MLVSS.h. In addition, if endogenous oxygen uptake is needed, oxygen consumption of microorganisms is measured without the addition of substrate. Microorganisms maintain their metabolic activities at minimum level by degrading own

cellular structure. As a result, minimum OUR values are measured. On the other hand, for the measurement of maximum OUR, samples should include easily biodegradable substrates. Under this condition, all bacteria are capable of degrading substrates and grow at maximum speed.

The respirometry test is well established and widely used nowadays for both research and at wastewater treatment plants. OUR measurements can be used in toxicity test for the detection of inhibitory streams. Respirometry is a very useful tool in measurement of toxicity detection since results are received quickly. In addition, by regular OUR tests at different places at the plant it is possible to follow changes in process performance. The measurements can be performed using simple equipment at wastewater treatment plants and it is relatively easy to apply and the data could be used for simpler characterization and process control compared to many other methods. Both batch tests and on-line measurements can be used depending on the purpose of application (Hagman and Jansen, 2007).

In addition, carbon dioxide (CO_2) production rate and cumulative carbon dioxide production of microorganisms can be measured by using a respirometer. In the flowing gas-static liquid respirometers, carbon dioxide production of microorganisms with respect to time is measured with a carbon dioxide sensor. Cumulative CO_2 production values show the amount of carbon dioxide produced and are reported in mg CO_2.

3. MATERIALS AND METHODS

As mentioned before, the present study was carried out in the second phase of a TÜBİTAK project (Project No. CAYDAG-111Y018, Microbial products and metal inhibition in biological systems). Both phases of the project are shown below.

Phase 1 – Former MSc. Thesis

Phase 1 determined the inhibitory effect of silver ion (Ag^+) on the performance of activated sludges operated at different COD/TKN ratios (10, 5 and 0). The research was mainly performed in three steps:

- Set-up and operation of activated sludge reactors named as R1, R2 and R3 which were operated at COD/TKN ratios of 10, 5 and 0, respectively,
- Determination of organic carbon removal and nitrification in these activated sludge reactors,
- Respiration inhibition tests with Ag^+ ion (Ayyıldız, 2013).

Phase 2 – The Current Thesis

Phase 2 is the subject of the present study. The aim was to determine the inhibitory effect of silver ion (Ag^+) on laboratory-scale activated sludges which were operated at the same carbon to nitrogen (COD/TKN) ratio of 10, but were fed with different organic substrates. Besides, the differences between the surface charge and hydrophobicity properties of these sludges were determined. The research was performed in six steps:

- Continuation of Phase 1 (operation of previously started activated sludge reactors R1, R2, R3),
- Conduction of an additional respirometry test with R3 sludge,
- Set-up and operation of new activated sludge reactors designated as Control Reactor (CR), Glucose Reactor (RG) and Peptone Reactor (RP),
- Determination of organic carbon and nitrogen removal in CR, RG and RP reactors,
- Respirometry tests with Ag^+ ion with sludges taken from the reactors in Phase 2 (CR, RG, RP)
- Surface charge and hydrophobicity analyses on CR, RG and RP sludges.

3.1. Operation of Activated Sludge Reactors

3.1.1. Activated Sludge

At the beginning of the study, 10 L of concentrated activated sludge was taken from the recycle line of the Paşaköy Advanced Biological Wastewater Treatment Plant. The main activated sludge reactor having a volume of 19 L was started up and 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 for a thesis study (Ayyıldız, 2013). Three reactors had a volume of 4 L (R1, R2 and R3) and one reactor had a volume of 9 L (CR). During that thesis study, CR reactor was 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. Daily 1/20 of the sludge was wasted from the reactor to have a sludge age of 20 days.

On the 27th of May 2013, glucose reactor (RG) and peptone reactor (RP) were started up with sludges taken from control reactor (CR). In the first phase, RG and RP reactors had a volume of 2 L and they were fed with Feed G and Feed P, respectively. The daily loading was the same as in the CR reactor. When these reactors reached steady-state conditions with respect to MLSS and MLVSS, their volume was increased to 4 L and they were fed under the same conditions. Daily 1/20 of the sludges was wasted from the reactors to have a sludge age of 20 days. All reactors are shown in Figure 3.1.

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

3.1.2. Preparation of Synthetic Wastewater

3.1.2.1. Feeding of Previously Started Reactors: Previously started reactors, R1, R2 and R3 were fed with Feed 1, Feed 2 and Feed 3, respectively. Composition of these feeds were given in a former MSc. thesis study (Ayyıldız, 2013).

3.1.2.2. Feeding of Control, Glucose and Peptone Reactors: In this study, three different synthetic wastewaters were prepared as "feeds" which had a different organic composition. All feeds had the same COD/TKN ratio of 10. Alkalinity was added to all feeds for nitrification.

As shown in Table 3.1., Feed 1 included glucose, acetate and peptone water as organic substances. Stock Feed 1 had a COD of 20000 mg/L and TKN of 2000 mg/L. Control Reactor (CR) was fed with this solution by using each time small volumes (460 mL).

Table 3.1. Composition of Stock Feed 1.

Feed 1	**Name**	**Formula**	**Molecular weight (g/mol)**	**Concentration (mg/L)**
Organics	D(+)-anhydrous glucose	$C_6H_{12}O_6$	180.2	5600
	Sodium acetate trihydrate	$CH_3COONa \cdot 3H_2O$	136.08	8000
	Peptone water			2000
Inorganics	Ammonium sulphate	$(NH_4)_2SO_4$	132.14	4000
	Sodium bicarbonate	$NaHCO_3$	84.01	2250
	Di-potassium hydrogen phosphate	K_2HPO_4	174.18	1000
	Potassium dihydrogen phosphate	KH_2PO_4	136.08	1000
	Magnesium sulphate	$MgSO_4$	120.37	1000
	Manganese (II) sulfate monohydrate	$MnSO_4.H_2O$	169.02	25
	Calcium sulphate dihydrate	$CaSO_4$	172.17	500
	Iron sulfate heptahydrate	$FeSO_4.7H_2O$	278.01	343

As shown in Table 3.2., Feed G included only glucose as an organic substance. Stock Feed G had a COD of 10000 mg/L and TKN of 1000 mg/L. Glucose Reactor (RG) was fed with this solution by using each time small volumes (400 mL).

Table 3.2. Composition of Stock Feed G.

Feed G	**Name**	**Formula**	**Molecular weight (g/mol)**	**Concentration (mg/L)**
Organics	D(+)-anhydrous glucose	$C_6H_{12}O_6$	180.2	9370,4
Inorganics	Ammonium sulphate	$(NH_4)_2SO_4$	132.14	4714
	Sodium bicarbonate	$NaHCO_3$	84.01	4500
	Di-potassium hydrogen phosphate	K_2HPO_4	174.18	1000
	Potassium dihydrogen phosphate	KH_2PO_4	136.08	1000
	Magnesium sulphate	$MgSO_4$	120.37	1000
	Manganese (II) sulfate monohydrate	$MnSO_4.H_2O$	169.02	25
	Calcium sulphate dihydrate	$CaSO_4$	172.17	500
	Iron sulfate heptahydrate	$FeSO_4.7H_2O$	278.01	343

As shown in Table 3.3., Feed P included only peptone water as an organic substance. Stock Feed P had a COD of 10000 mg/L and TKN of 1000 mg/L. Peptone Reactor (RP) was fed with this solution by using each time small volumes (400 mL).

Table 3.3. Composition of Stock Feed P.

Feed P	**Name**	**Formula**	**Molecular weight (g/mol)**	**Concentration (mg/L)**
Organics	Peptone Water			16051
Inorganics	Ammonium sulphate	$(NH_4)_2SO_4$	132.14	283
	Sodium bicarbonate	$NaHCO_3$	84.01	4500
	Di-potassium hydrogen Phosphate	K_2HPO_4	174.18	1000
	Potassium dihydrogen phosphate	KH_2PO_4	136.08	1000
	Magnesium sulphate	$MgSO_4$	120.37	1000
	Manganese (II) sulfate monohydrate	$MnSO_4.H_2O$	169.02	25
	Calcium sulphate dihydrate	$CaSO_4$	172.17	500
	Iron sulfate heptahydrate	$FeSO_4.7H_2O$	278.01	343

3.1.3. Monitoring of Activated Sludge Reactors

All activated sludge rectors were monitored to control the COD removal efficiencies and physical conditions, such as pH and temperature. For this reason, COD, MLSS, MLVSS and pH measurements were done regularly.

Reactors were fed on Mondays, Wednesdays and Fridays while the daily loading rate was as 500 mg COD/L.day and 50 mg TKN/L.day. The sludge age in reactors was controlled by wasting some amount of sludge on feeding days. By doing this, the MLVSS values could be kept at a nearly constant level. Reactor operation conditions can be seen in **Appendix A.**

3.1.4. Surface Charge Measurements

Surface charge (SC) analyses were done to determine the differences between the reactors which were fed with different organic substrates. The analyses were done with the samples taken from the reactors before and after feeding.

For these analyses the colloidal titration method was used. Sludge samples were taken from the reactors and washed twice in order to remove residual substrate. Then, 10 mL sample was diluted to 100 mL with deionized water and put into an Erlenmeyer flask. The pH was adjusted to 7. 5 mL 0.001 N polybrene solution and a few drops of Toluidine blue indicator were added. The solution was titrated with PVSK solution until the color changed from blue to pink/purple. When color change was observed, titration was ended and the volume of PVSK was recorded. The same steps were followed with the same amount of polybrene and deionized water as blank. All analyses were done in duplicates and the average values were reported. The SC of sludge was calculated by using the following formula:

$$\text{Surface Charge (meqv/g MLSS)} = \frac{(A-B) \times N \times 1000}{\text{mL of sample} \times \text{mg/L MLSS}} \times 1000 \qquad (3.1)$$

where A is the mL of used PVSK for sample, B is the mL of used PVSK for blank, N is the normality of PVSK.

The color change during surface charge measurements is shown in Figure 3.2 and Figure 3.3.

Figure 3.2. Color change in the blank before and after titration.

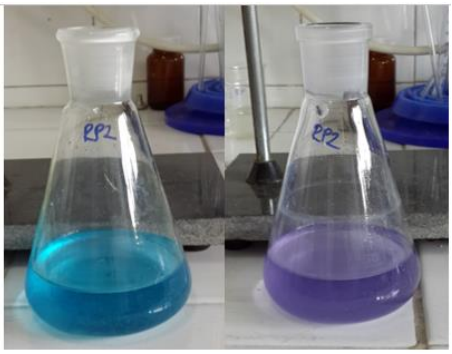

Figure 3.3. Color change in the sample before and after titration.

3.1.4.1. Materials used in Surface Charge Measurements:

Polyvinyl Sulfuric Acid Potassium Salt Solution: Polyvinyl Sulfuric Acid Potassium Salt (PVSK) solution was used for surface charge analysis of activated sludges as an anionic standard. The brand of this chemical was "Acros Organics polyvinyl sulfuric acid potassium salt". The stock solution was prepared as 0.5 g/L to have 0.001 N PVSK solution. The solution was standardized after each preparation by using a zephiramine solution. It was then stored at 4°C.

Zephiramine Solution: Zephiramine solution was used for the standardization of PVSK solution. 0.505 g of zephiramine was weighed and dissolved in water to make 500 mL solution. The brand of this chemical was "TCI Europe-Tetradecyldimethylbenzylammonium chloride". The stock solution was freshly prepared for the standardization of PVSK and not stored.

Polybrene Solution: Polybrene solution was used for surface charge analysis of activated sludges as a cationic standard. The brand of this chemical was "Sigma-Aldrich polybrene-hexadimethrine bromide". The stock solution was prepared as 0.2 g/L to have a 0.001 N Polybrene solution.

Toluidine Blue Indicator: Toluidine blue (TB) indicator was used in surface charge analysis to determine the end-point. The brand of this chemical was "Merck Toluidine blue O for microscopy". 0.1 g of TB was weighed and dissolved in water to make 100 mL solution.

3.1.5. Hydrophobicity Measurements

Hydrophobicity analyses were done on sludges to determine the differences between the reactors which were fed by different organic substrates. These analyses were done on the same samples as used in surface charge (SC) analyses. In hydrophobicity measurement, the octane adhesion test method (a MATH test) was used. The absorbance of the sample was initially adjusted to nearly 0.3 at 600 nm. Then, 10 mL of sample was put in a 50 mL tube and 4 mL n-octane solution was added. The suspension was vortexed for 2 minutes and settled for 10 minutes for phase separation. The sample was withdrawn from the aqueous phase and the optical density (OD_{600}) was measured at 600 nm. It was reported as the final optical density. All analyses were done in duplicates and the average values were reported. The hydrophobicity of sludge was calculated by using the following formula:

$$\text{Hydrophobicity (\%)} = (1 - (Abs_{final} / Abs_{initial})) \times 100 \quad (3.2)$$

The separation of phases after n-octane addition and vortexing is shown in Figure 3.4.

Figure 3.4. Phase separation after octane addition and vortexing.

3.1.5.1. Materials used in Hydrophobicity Measurements:

n-Octane: n-Octane was used as a hydrocarbon in hydrophobicity measurements. The brand of this chemical solution was “Merck n-octane for synthesis”.

3.1.6. Respirometry Tests

Respirometry tests were done 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”.

In respirometry tests, a flowing gas-static liquid respirometer “Columbus Oxymax ER-10 respirometer” and OLS200 Grant Shaker were used. The respirometer has two sensors for the measurement of oxygen and carbon dioxide. The oxygen sensor measures oxygen consumption with electrochemical methods. Carbon dioxide sensor measures carbon dioxide production with a single beam, non-dispersive IR spectrophotometer. In the respirometer, measurements are performed in a closed gas sensing loop. Throughout the measurements, the gas present in the headspace of the test chamber is circulated through the sensor and back to the test chamber for a fixed period of time. 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. Then, volume of gas consumed or produced 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 given in mg O_2/min or as an accumulated (total in mg) value of oxygen consumed from the beginning of the experiment. Samples are continuously aerated with adjustable air flow (100 mL/min to 1500 mL/min) except for the short time interval when a particular sample is being measured by the gas analyzer. For the control of data collection, respirometer communicates with host computer and a software program is used to arrange number of test samples, calibrate gas sensor and measure test chamber head space volume. ER-10 Respirometer is capable of taking

measurements directly up to 10 different test samples and give the real-time graphical data representation. The diagram of the respirometer is shown in Figure 3.5.

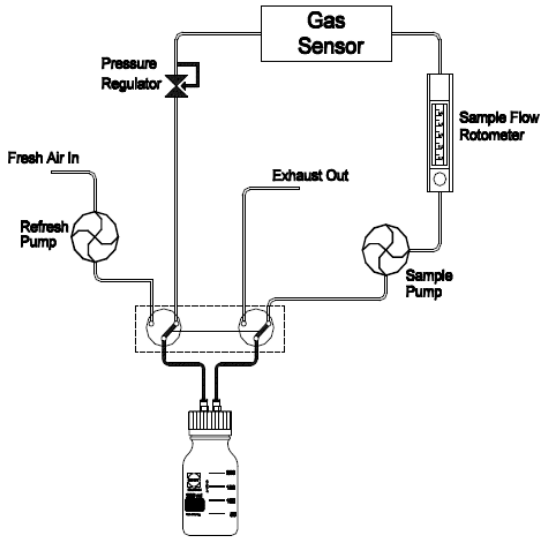

Figure 3.5. The diagram of ER-10 Respirometer.

In the respirometer, 10 different respirometric chambers are present. Generally, in all tests three different sludges (from CR, RG and RP reactors) were used in the same test in order to determine the differences. For this purpose, 20 mL sludge sample, necessary feed and Ag^+ were added to the test chambers. In order to observe the inhibition caused by Ag^+, various doses of Ag was added. In addition, in same tests the nitrification inhibitor (ATU) was added to measure carbonaceous oxygen consumption and carbon dioxide production only. In all chambers the final liquid volume was 100 mL. pH of each chamber was adjusted to 7.5, then all chambers were put into the shaker at 25^{o}C, and shaken at 120 rpm for nearly 22 hours. The configuration of the test is shown in Figure 3.6.

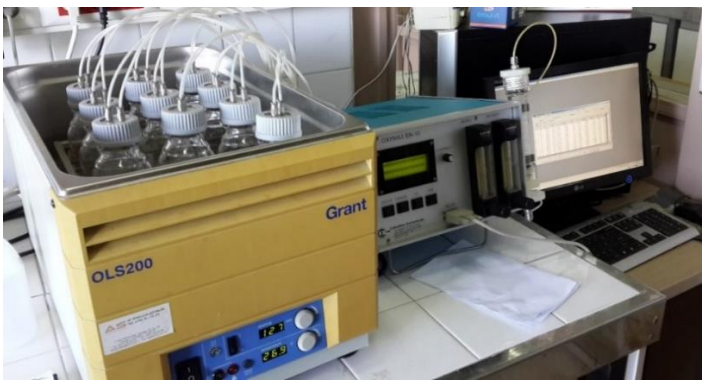

Figure 3.6. Configuration of Respirometric Test.

In respiration tests, the performance of chambers was also monitored analytically. pH and COD were measured at the start and end of a test. Moreover, for each sludge used in the test; MLSS, MLVSS, surface charge and hydrophobicity analyses were carried out at the start of a respirometry test.

From respirometry tests, following data were obtained with respect to time:

- Oxygen uptake rate: instantaneous oxygen uptake rate (mg/min),
- Cumulative oxygen uptake: the total amount of oxygen uptake in a test (mg),
- Carbon dioxide production rate: instantaneous carbon dioxide production rate (mg/min),
- Cumulative carbon dioxide production: the total amount of carbon dioxide production in a test (mg).

The results were presented in the form of four figures:

- Oxygen uptake rate,
- Cumulative oxygen uptake,
- Carbon dioxide production rate,
- Cumulative carbon dioxide production.

All raw data belonging to respirometry tests are presented in **Appendix B.** The notations used in these figures are presented in Table 3.4.

Table 3.4. Notations in respirometric figures.

NOTATION	EXPLANATION
Ch	Number of respirometric test chamber
CR, RG, RP	Activated sludge samples taken from the control (CR), glucose (RG) and peptone (RP) reactors
Feed	Addition of feed solution (Feed 1, G or P)
ATU	Addition of the nitrification inhibitor, ATU to test chamber
mg/L Ag	Concentration of Ag metal in the test chamber
CR, RG, RP Sludge	Measurement of endogenous respiration in CR, RG or RP sludges (only activated sludge sample and deionized water)

3.1.6.1. Materials used in Respirometry Tests:

Silver Used in Respirometry Tests: In the respiration tests with Ag, a commercial Ag solution was used. For this purpose, Fluka Analytical 12818 Silver Standard for ICP solution was purchased. This standard solution had a concentration of 1000 mg/L Ag in 2% nitric acid. Ag is found as Ag^+ ion (free silver ion) in this solution. In experiments, this standard solution was diluted in order to reach the desired concentration.

Nitrification Inhibitor: In respirometry tests a nitrification inhibitor was used to differentiate carbonaceous oxygen demand ($C\text{-}O_2$) from the nitrogenous oxygen demand ($N\text{-}O_2$). $C\text{-}O_2$ arises due to organic carbon removal and $N\text{-}O_2$ arises due to nitrification.

N-allylthiourea (ATU) was used in respirometry tests as a nitrification inhibitor. The brand of this chemical was 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 0.5 mL was added to respirometry chambers to reach a final concentration of 11.6 mg/L ATU (10^{-4} mol/L). 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. Since it is not stable for more than 2 weeks (APHA et al., 2004), ATU stock solution was prepared weekly.

3.1.6.2. Processing of Raw Respirometric Data: Using raw respirometric data, cumulative total oxygen uptake ($T\text{-}O_2$) and cumulative carbonaceous oxygen uptake ($C\text{-}O_2$), cumulative carbon dioxide production ($T\text{-}CO_2$) and cumulative carbonaceous carbon dioxide production ($C\text{-}CO_2$) were found. Also, cumulative nitrogenous oxygen uptake ($N\text{-}O_2$) and cumulative nitrogenous carbon dioxide production ($N\text{-}CO_2$) could be calculated by using these data.

Nitrogenous O_2 and nitrogenous CO_2 values were calculated as follows:

$$N\text{-}O_2 = T\text{-}O_2 - C\text{-}O_2 \tag{3.3}$$

$$N\text{-}CO_2 = T\text{-}CO_2 - C\text{-}CO_2 \tag{3.4}$$

Figure 3.7. and Figure 3.8. show an example on how raw data are processed. In these figures, the oxygen consumption due to organic carbon removal and nitrification (C-O_2+N-O_2) is presented as total oxygen consumption (T-O_2). In some respirometric chambers, a nitrification inhibitor was added in order to determine C-O_2 separately. By using these results, N-O_2 was obtained from the difference.

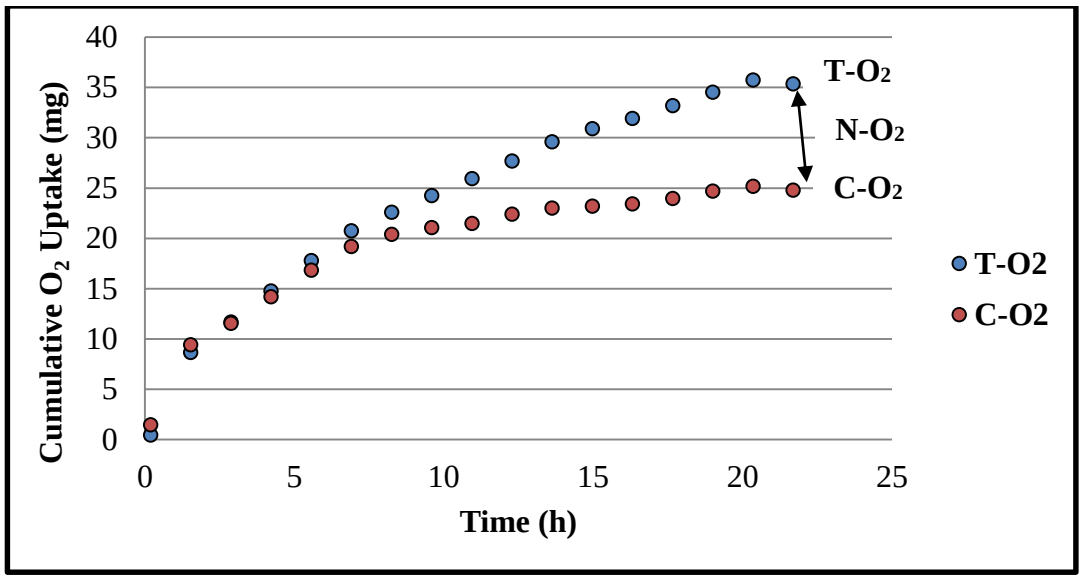

Figure 3.7. Example of N-O_2 calculation from raw respirometric data.

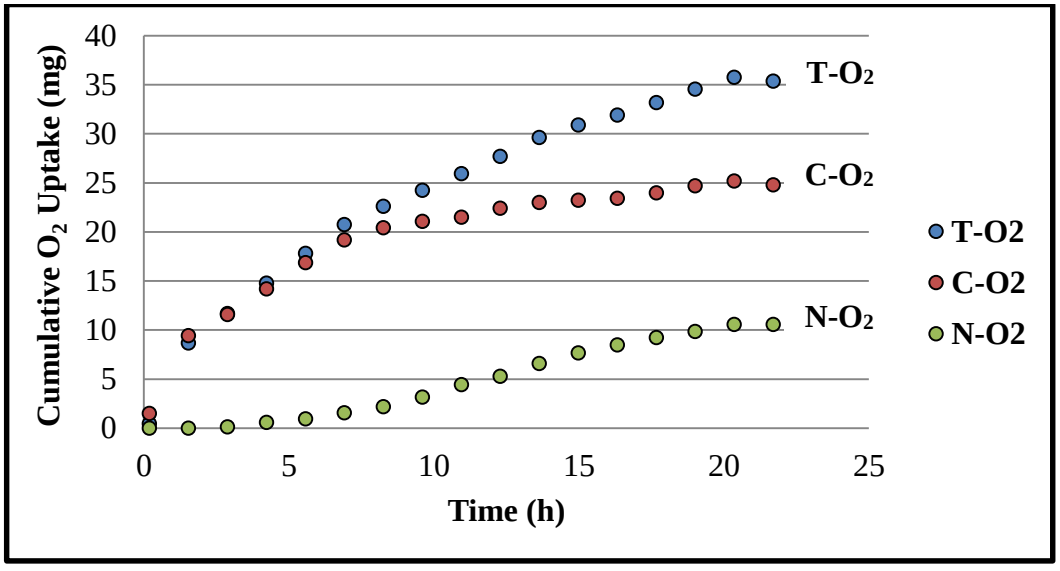

Figure 3.8. Example of graphical presentation of N-O_2.

3.1.7. Sorption of Ag onto Sludge

Sorption tests were done in order to determine the sorption capacity of silver on the surface of activated sludge. Within the scope of the project, the results of these tests will be used in MINTEQA2 program to determine the speciation of the silver metal. The purpose of these analyses was to observe the differences between the adsorption capacities of activated sludges which were fed with different organic substances.

Sorption tests were carried out with the sludge samples taken from CR, RG and RP reactors. Samples were washed twice in order to remove substrate. 20 mL sludge was put in 100 mL respirometry chamber and different concentrations of Ag (1, 2, 3, 4 and 5 mg/L for these analyses) were added. In the chambers the final liquid volume was 100 mL and they were shaken for 1 hour. It was assumed that after 1 hour, adsorption reached equilibrium according to previous studies (Çeçen et al., 2010). After 1 hour, samples were taken to measure total and soluble silver.

3.2. Analytical Methods

Analyses in experiments were done according to the Standard Methods for the Examination of Water and Wastewater (APHA et al., 1999).

3.2.1. MLSS and MLVSS Analysis

For the MLSS analysis, 10 mL sample was filtered through filter paper (Sartorius Stedim Biotech Glassfiber Prefilter 0.45 μm) and the residue on the filter paper was dried for one hour at 103^{o}C in the FN 500 oven. For MLVSS analysis, the residue was ignited after MLSS analysis for 30 minutes at 550^{o}C in the Protherm muffle furnace. All MLSS and MLVSS analysis were done in duplicates and the averages were reported.

3.2.2. Chemical Oxygen Demand (COD) Analysis

COD analyses were done to determine the organic carbon removal in reactors and respirometry tests. The method was the dichromate closed reflux and colorimetric method.

In this method, organic matter is oxidized by potassium dichromate under strongly acidic conditions. In the COD analysis, 2.5 mL sample was put in 10 mL COD tube and 1.5 mL potassium dichromate ($K_2Cr_2O_7$) solution and 3.5 mL sulfuric acid (H_2SO_4) solutions were added. There were Ag_2SO_4 as catalyst and $HgSO_4$ for preventing chloride interference in $K_2Cr_2O_7$ solution and H_2SO_4 solution, respectively. Samples were refluxed for 2 hours at 150^oC in the ECO 25 Thermoreactor COD digester. The digested samples were measured colorimetrically at 600 nm with the Hach DR3900 Spectrophotometer. The calibration curves were prepared by using Potassium Hydrogen Phthalate (KHP) solution every time the $K_2Cr_2O_7$ solution was prepared. The analyses were done in triplicates and the averages were reported during the study.

3.2.3. pH Analysis

WTW Inolab-1 pH meter was used for pH measurements. The calibration of the pH probe was done every 2 or 3 weeks by using standard buffer solutions having pH values of 4 and 7.

3.2.4. NH_4-N Analysis

NH_4-N analyses were done in the case of Reactor 3. The Nessler Method was used in these analyses. For this purpose, the Method 8038 in the Hach Water Analysis Handbook, 5th edition was followed and Hach DR3900 Spectrophotometer was used. After necessary dilutions, 3 drops of Hach Mineral stabilizer, 3 drops of Hach Polyvinyl alcohol dispersing agent and 1 mL Merck Nessler reagent were added to 25 mL sample and 25 mL deionized water as the blank. After one minute, NH_4-N concentration of the sample was read in mg/L by using the spectrophotometer.

3.2.5. Metal Analysis

Hot Plate Digestion method was used to measure Ag concentration in samples taken from sorption analyses. In this method, 10 mL sample was put in a beaker and 5 mL HNO_3 and 2 mL H_2O_2 were added. The duration of digestion was 4 hours. After 4 hours, samples were put in 10 mL volumetric flasks and the final volume was adjusted to 10 mL.

Concentration of Ag was measured with PERKIN ELMER AAnalyst 300 Atomic Adsorption Spectrometry (AAS). The results of the tests are given in the Section "Results and Discussion".

3.2.5.1. Sensitivity of Metal Analysis: Sensitivity tests for different silver concentrations were carried out in order to determine the errors coming from preparation of samples. For this purpose, 1 and 4 mg/L Ag concentrations were used. Ag concentrations were measured with PERKIN ELMER AAnalyst 300 Atomic Adsorption Spectrometry (AAS). Results are presented in Table 3.5.

Table 3.5. Results of silver measurements.

Number of sample	**For 1 mg/L Ag addition**	**For 4 mg/L Ag addition**
1	1.006 mg/L	3.664
2	0.992 mg/L	3.797
3	0.935 mg/L	3.856
4	0.994 mg/L	3.668
5	1.007 mg/L	3.819
Average	**0.99**	**3.76**

This table shows the average results of measurements for 1 mg/L and 4 mg/L as 0.99 mg/L and 3.76 mg/L, respectively. Standard deviations were calculated as 0.027 and 0.08 for 1 mg/L and 4 mg/L, respectively. These results indicate that there was a slight error in the preparation of samples.

4. RESULTS AND DISCUSSION

4.1. Operation of Activated Sludge Reactors

As explained in "Materials and Methods", in order to investigate the removal of organic carbon and nitrification in activated sludge reactors which were fed with different organic substrates, three different reactors have been operated. The results of the operation period are given in the following sections.

Within the scope of the TÜBİTAK project, these three reactors (CR, RG and RP) and the former reactors (R1, R2 and R3) were monitored in terms of EPS composition and production within the scope of a Ph.D. thesis. The Ph.D. thesis investigates the relationship between metal inhibition and microbial products in biological systems and it is still in progress (Geyik, 2014).

4.1.1. Removal of Organic Carbon in Reactors

Organic carbon removal in the reactors was measured with COD analysis. For all reactors, influent COD, effluent COD and removal efficiencies are presented in **Appendix A** in detail. Influent COD and effluent COD show the values measured at the initial condition and at the end of a semi-continuous run, respectively.

In the present thesis, the operation period for CR, RG and RP reactors started at 1st July 2013 and lasted to 12th September 2014. In this period of 438 days, many semi-continuous runs were monitored and in parallel to this 20 respirometric tests were carried out. All raw data are shown in **Appendix A** and **Appendix B,** respectively. Additionally, as mentioned before, the former reactors (R1, R2 and R3) were also operated. Raw data of these reactors are also tabulated in **Appendix A.**

Control Reactor (CR) was fed with Feed 1 which included glucose, acetate and peptone water as organic substances. Figure 4.1. shows the influent and effluent COD during the operation of CR. According to the results, it can be said that CR reactor has a COD removal

efficiency of approximately 85-90%. Average COD influent and effluent were calculated as 965±69 mg/L and 103±48 mg/L, respectively.

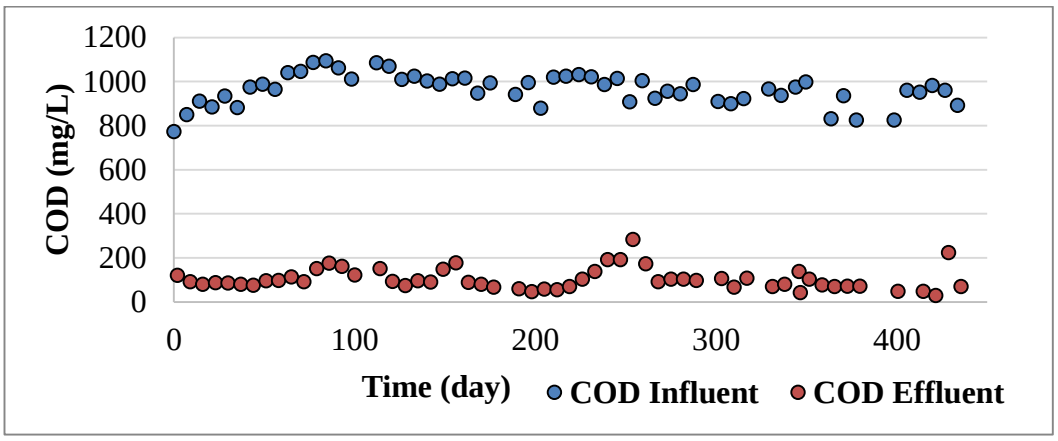

Figure 4.1. COD values in the semi-continuous operation of the Control Reactor (CR).

Glucose Reactor (RG) was fed with Feed G which included only glucose as an organic substance. Figure 4.2. shows the influent and effluent COD in RG reactor. According to the results, RG reactor has a COD removal efficiency higher than 90%. Average COD influent and effluent were calculated as 844±77 mg/L and 43±22 mg/L, respectively.

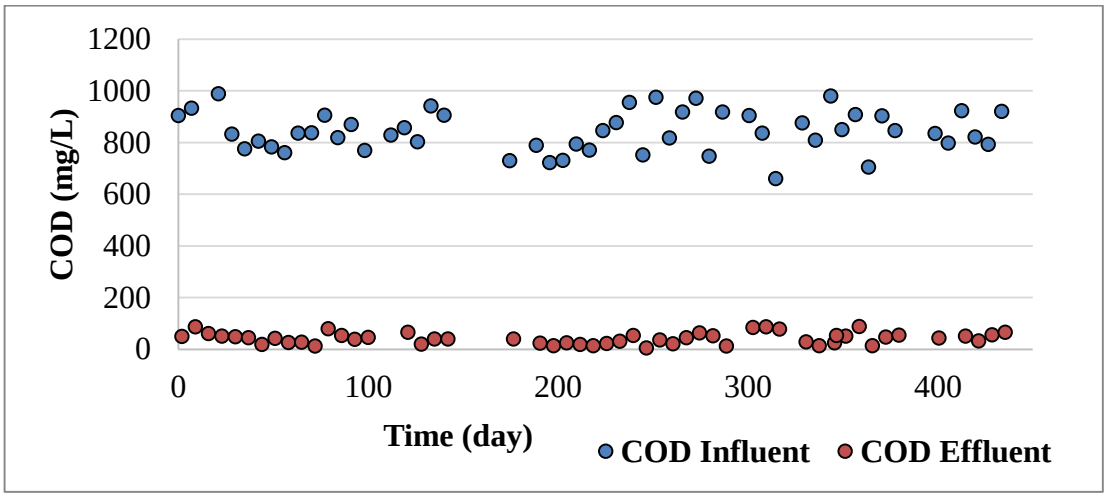

Figure 4.2. COD values in the semi-continuous operation of the Glucose Reactor (RG).

Peptone Reactor (RP) was fed with Feed P which included only peptone water as an organic substance. Figure 4.3. shows the influent and effluent COD in RP reactor. According

to the results, it can be said that RP reactor had a COD removal efficiency of approximately 85-90%. Average COD influent and effluent were calculated as 848±101 mg/L and 74±33 mg/L, respectively.

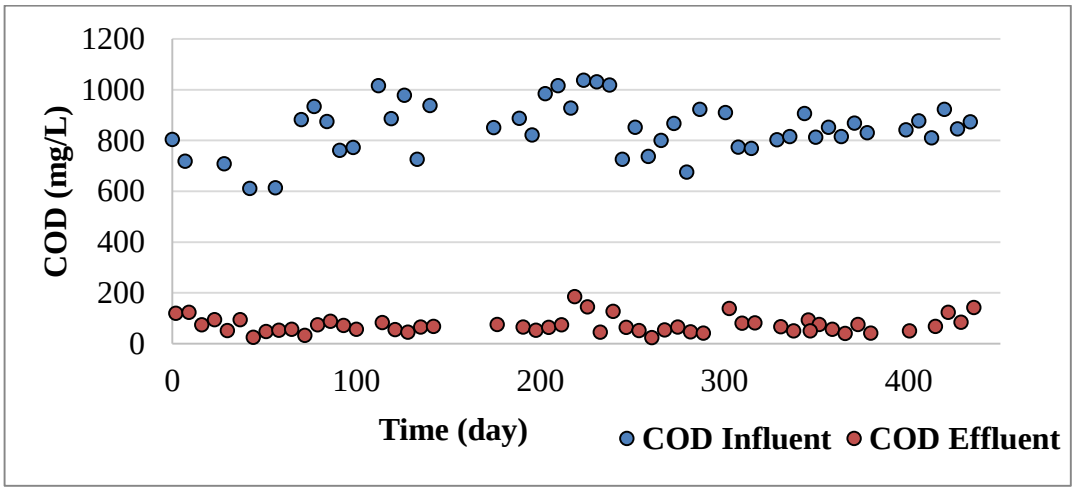

Figure 4.3. COD values in the semi-continuous operation of the Peptone Reactor (RP).

When all results are taken in consideration, it can be seen that the organic carbon removal in these three reactors was close to each other and the removal efficiencies were high. In addition, all reactors were operated under steady-state conditions.

4.1.2. pH Profiles in Reactors

pH measurements were done at the start and at the end of each semi-continuous feeding period in order to control the reactors. Additionally, while measuring pH, temperature measurements were done in reactors. The pH in the reactors are shown in the following figures.

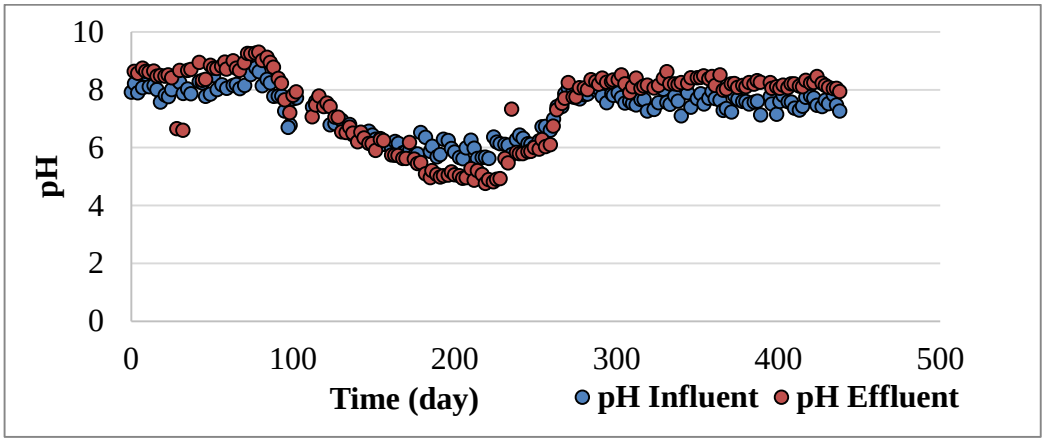

Figure 4.4. pH profiles in the Control Reactor (CR).

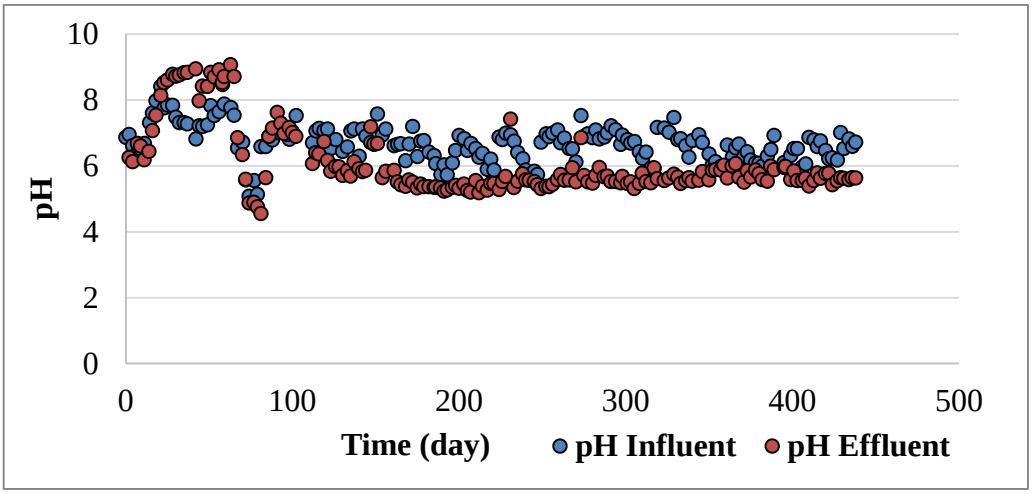

Figure 4.5. pH profiles in the Glucose Reactor (RG).

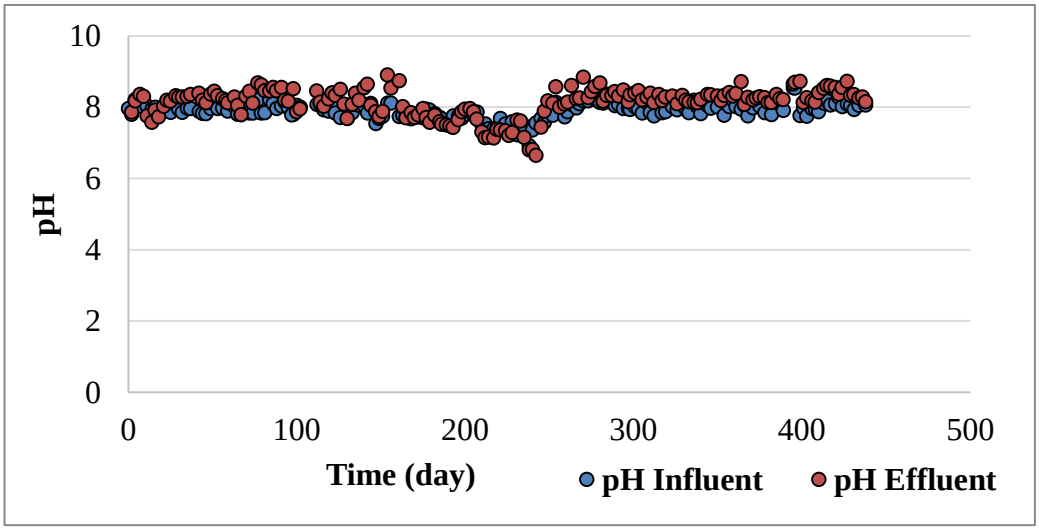

Figure 4.6. pH profiles in the Peptone Reactor (RP).

4.1.3. MLSS and MLVSS Profiles in Reactors

MLSS and MLVSS analyses were done to get information about the biomass concentrations in the reactors. Control of biomass concentration is important, because activated sludge samples were regularly taken from these reactors for respirometry tests and EPS extractions. The MLSS and MLVSS values in the reactors are shown in following figures. Average MLSS concentrations in CR, RG and RP were found as 4236 mg/L, 3875 mg/L and 4946 mg/L, respectively. Additionally, average MLVSS concentrations in CR, RG and RP were found as 3101 mg/L, 3212 mg/L and 3129 mg/L, respectively.

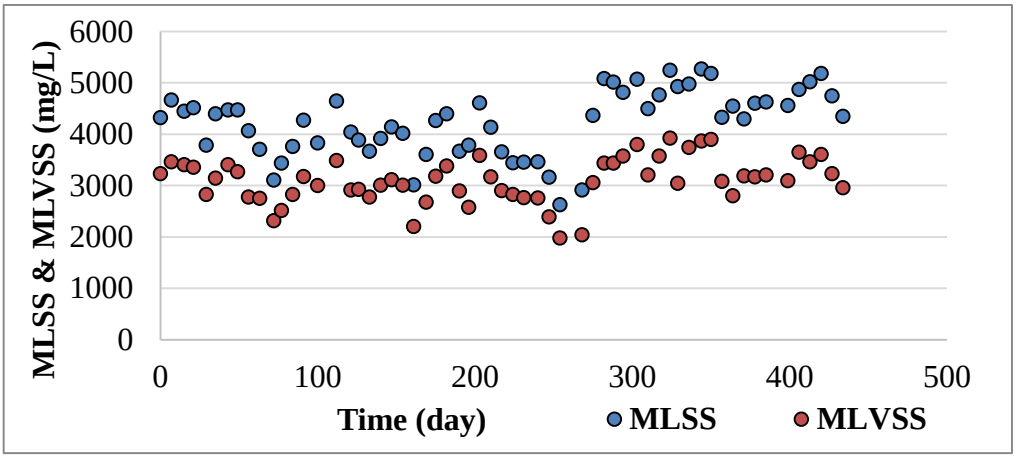

Figure 4.7. MLSS and MLVSS profiles in the Control Reactor (CR).

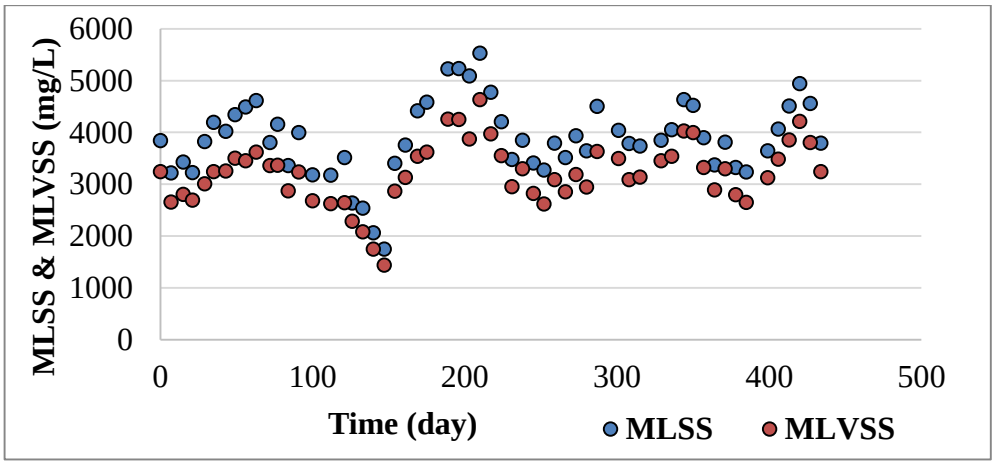

Figure 4.8. MLSS and MLVSS profiles in the Glucose Reactor (RG).

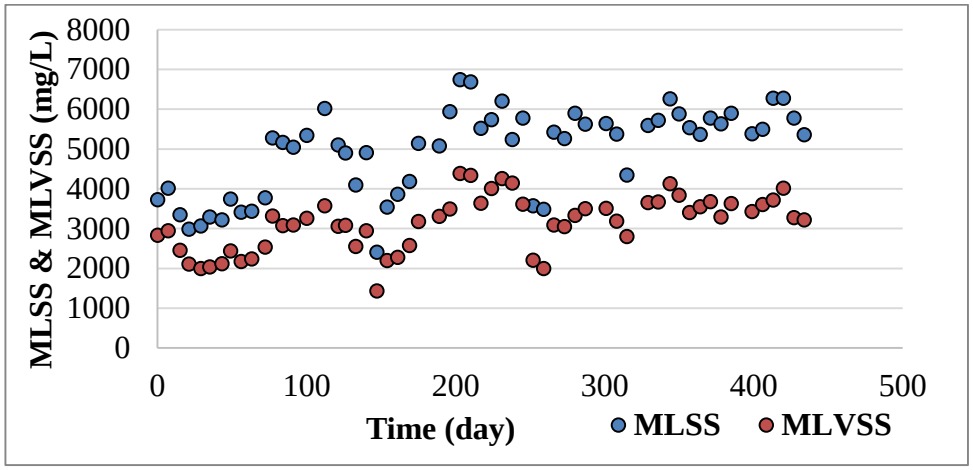

Figure 4.9. MLSS and MLVSS profiles in the Peptone Reactor (RP).

4.1.4. Calculation of Loading and Removal Rates in Reactors

The F/M ratios were calculated under the conditions of each semi-continuous run. For this calculation, the concentration of feed stock, concentration of microorganism in the reactor and the duration of run were used. As an example, the F/M ratio in RG was calculated as follows:

Substrate in stock feed, S = 10000 mg COD/L

Duration of a semi-continuous run = 1.80 day

Flow rate, Q = 400 mL/1.80 day = 0.22 L/day (Addition of 400 mL of stock feed at the start of each run)

Biomass concentration in the reactor, X = 3190 mg MLVSS/L

Volume of the reactor, V = 4 L

$$\text{F/M Ratio} = \frac{Q \times S_0}{V \times X} = \frac{0.22\ \text{L/day} \times 10000\ \text{mg COD/L}}{4\ \text{L} \times 3190\ \text{mg MLVSS/L}} \qquad (4.1)$$

$$= 0.17\ \text{mg COD / mg MLVSS.day}$$

Also, the initial substrate per biomass (S_0/X_0 ratio) at the start of each semi-continuous run was calculated. For this calculation, the initial substrate and MLVSS concentration were used. The results are given in **Appendix A.**

As an example, if the initial concentration at the start of a semi-continuous run in a reactor was 1016 mg/L COD and the initial biomass concentration was 3570 mg/L, the S_0/X_0 ratio was as follows:

$$\frac{S_0}{X_0} = \frac{1016 \text{ mg COD/L}}{3570 \text{ mg MLVSS/L}} = 0.28 \text{ mg COD/mg MLVSS} \quad (4.2)$$

Additionally, the specific substrate removal rate (q) was calculated. For this calculation, F/M ratio and percent COD removal were used. As an example, if the initial and final COD concentrations were 972 mg/L and 64 mg/L, respectively; the percent removal was calculated as follows:

$$\frac{S_0\text{-}S}{S} = \frac{972 \text{ mg COD/L} - 64 \text{ mg COD/L}}{972 \text{ mg COD/L}} = 0.93 \times 100 = 93\ \% \quad (4.3)$$

Then, if the F/M ratio was 0.17 mg COD/mg MLVSS.day, q was then calculated as follows:

$$q = 0.17 \text{ mg COD / mg MLVSS.day} \times 0.93 = 0.16 \text{ mg COD / mg MLVSS.day} \quad (4.4)$$

4.1.5. Loading and Removal Rates in Previously Started Reactors (R1, R2, R3)

Loading rates (F/M) and specific substrate removal rates (q) in previously started reactors are shown in Figure 4.10., Figure 4.11. and Figure 4.12. These figures show the data belonging to the period of the present study. In these three figures t=0 shows 1st July 2013. The average F/M ratios in R1 and R2 sludges were found as 0.19±0.05 and 0.09±0.02 mg COD/mg MLVSS.day, respectively. Also, the average specific removal rates (q) of these sludges were found as 0.17±0.05 and 0.08±0.02 mg COD/mg MLVSS.day. For R3 sludge, the average F/M and q were found as 0.26±0.1 and 0.22±0.09 mg NH_4-N/mg MLVSS.day, respectively.

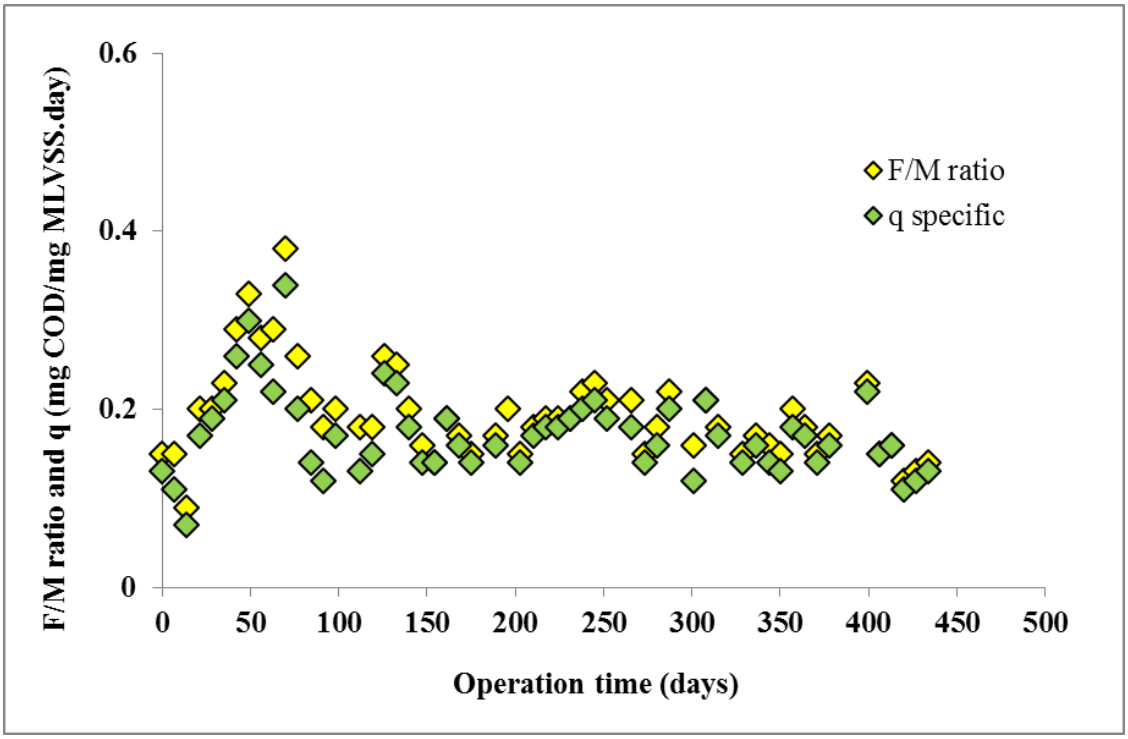

Figure 4.10. Loading rate (F/M) and specific removal rate (q) profiles in R1 operation.

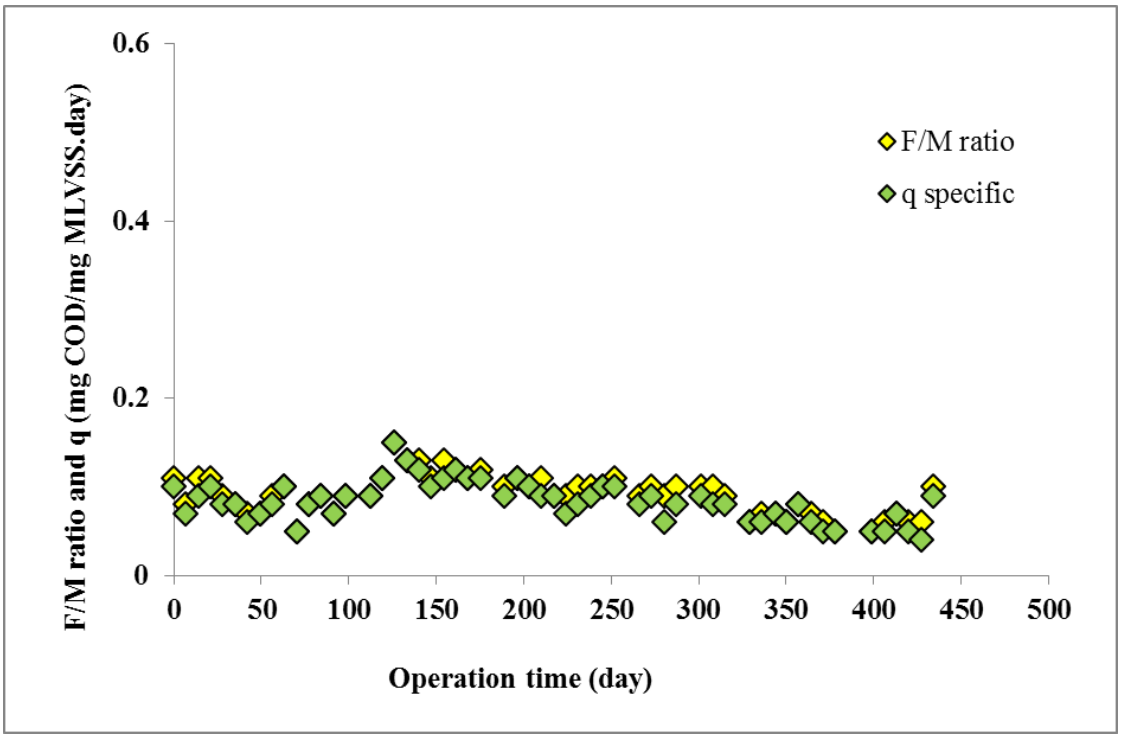

Figure 4.11. Loading rate (F/M) and specific removal rate (q) profiles in R2 operation.

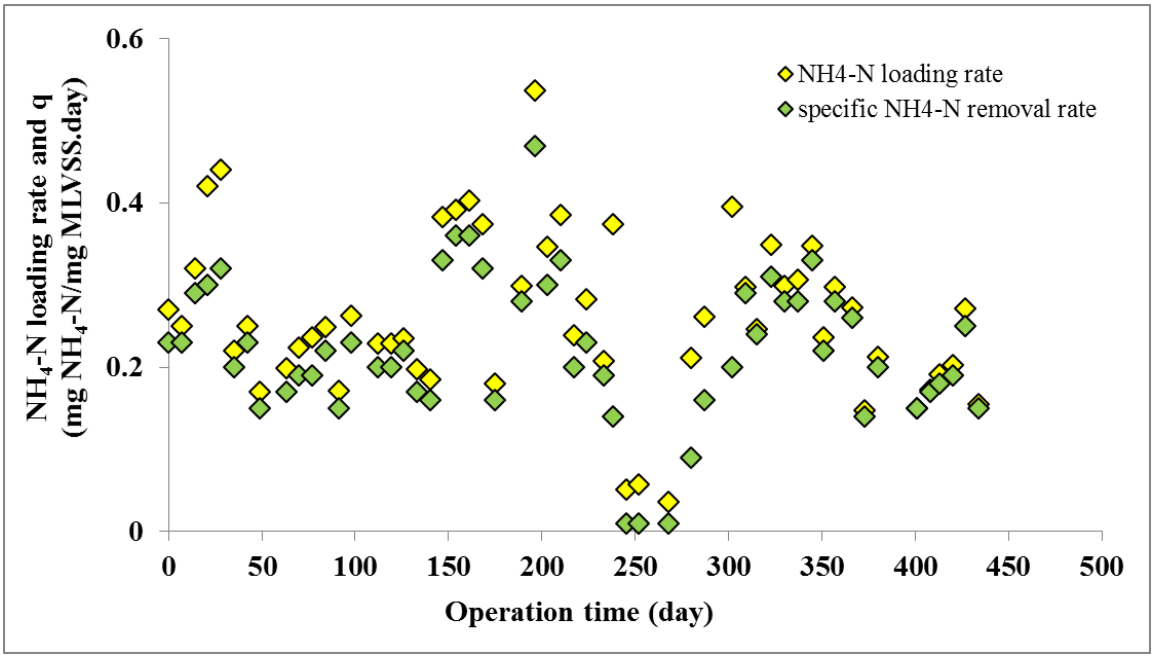

Figure 4.12. Loading rate (F/M) and specific removal rate (q) profiles in R3 operation.

4.2. Respirometry Tests

The respirometry tests constitute the major part of this study. These tests were carried out for two main purposes. The first aim was to determine the extent of organic carbon removal and nitrification. The second aim was to observe the inhibitory effect of silver on different types of activated sludge. All respirometric tests are listed in chronological order in Table 4.1. These tests were begun on 6th November 2013 and ended on 10th November 2014.

Table 4.1. Properties of respirometric tests shown in chronological order.

Test No:	Date	Reactor Name	Feed	THEORETICAL VALUES			ANALYTICAL VALUES				
				Feed Concentration	Ag Concentration (mg/L)	ATU (mg/L)	Initial SC (meqv/g SS)	Initial Hydro. (%)	Initial COD (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
1	06.11.2013	Control Reactor	Feed 1	500 mg/L COD	0	0	not measured	not measured	not measured	not measured	not measured
		Glucose Reactor	Feed G	500 mg/L COD							
		Peptone Reactor	Feed P	500 mg/L COD							
2	13.11.2013	Control Reactor	Feed 1	500 mg/L COD	0	0	not measured	not measured	616	927	724
		Glucose Reactor	Feed G	500 mg/L COD					592	1158	939
		Peptone Reactor	Feed P	500 mg/L COD					607	1077	680
3	20.11.2013	Control Reactor	Feed 1	500 mg/L COD	0	11.6	not measured	not measured	498	853	673
		Glucose Reactor	Feed G	500 mg/L COD					463	792	653
		Peptone Reactor	Feed P	500 mg/L COD					463	1001	643
4	21.01.2014	Control Reactor	Feed 1	500 mg/L COD	3	0	not measured	not measured	410	682	575
		Glucose Reactor	Feed G	500 mg/L COD					454	961	769
		Peptone Reactor	Feed P	500 mg/L COD					459	1259	852
5	03.07.2014	Control Reactor	Feed 1	500 mg/L COD	5	0	-0.144	48	446	1120	813
		Glucose Reactor	Feed G	500 mg/L COD			-0.108	55	336	934	802
		Peptone Reactor	Feed P	500 mg/L COD			-0.057	69	370	1394	927

SC: Surface Charge
Hydro: Hydrophobicity

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

Test No:	Date	Reactor Name	Feed	THEORETICAL VALUES			ANALYTICAL VALUES				
				Feed Concentration	Ag Concentration (mg/L)	ATU (mg/L)	Initial SC (meqv/g SS)	Initial Hydro. (%)	Initial COD (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
6	08.07.2014	Control Reactor	Feed 1	500 mg/L COD	4	0	-0.108	58	418	897	711
		Glucose Reactor	Feed G	500 mg/L COD			-0.132	65	387	788	688
		Peptone Reactor	Feed P	500 mg/L COD			-0.064	73	501	1286	845
7	10.07.2014	Control Reactor	Feed 1	500 mg/L COD	3	0	-0.137	64	432	960	744
		Glucose Reactor	Feed G	500 mg/L COD			-0.103	74	359	954	819
		Peptone Reactor	Feed P	500 mg/L COD			-0.068	80	414	1358	907
8	15.07.2014	Control Reactor	Feed 1	500 mg/L COD	0	11.6	-0.098	60	417	1131	870
		Glucose Reactor	Feed G	500 mg/L COD			-0.130	71	411	979	833
		Peptone Reactor	Feed P	500 mg/L COD			-0.064	57	337	1518	1001
9	05.08.2014	Control Reactor	Feed 1	500 mg/L COD	2	11.6	-0.073	53	410	1360	1027
		Glucose Reactor	Feed G	500 mg/L COD			-0.100	51	428	1047	885
		Peptone Reactor	Feed P	500 mg/L COD			-0.048	71	353	1472	1036
10	07.08.2014	Control Reactor	Feed 1	500 mg/L COD	3	0	-0.094	74	397	1014	780
		Glucose Reactor	Feed G	500 mg/L COD			-0.098	38	408	1075	883
		Peptone Reactor	Feed P	500 mg/L COD			-0.059	75	340	1404	910

SC: Surface Charge
Hydro: Hydrophobicity

Table 4.3. Properties of respirometric tests shown in chronological order (continued).

Test No:	Date	Reactor Name	Feed	THEORETICAL VALUES			ANALYTICAL VALUES				
				Feed Concentration	Ag Concentration (mg/L)	ATU (mg/L)	Initial SC (meqv/g SS)	Initial Hydro. (%)	Initial COD (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
11	12.08.2014	Control Reactor	Feed 1	500 mg/L COD	4	11.6	-0.108	71	432	1060	824
		Glucose Reactor	Feed G	500 mg/L COD			-0.083	55	479	1075	927
		Peptone Reactor	Feed P	500 mg/L COD			-0.081	71	486	1215	801
12	19.08.2014	Control Reactor	Feed 1	500 mg/L COD	5	11.6	-0.087	54	403	1139	817
		Glucose Reactor	Feed G	500 mg/L COD			-0.091	53	398	890	763
		Peptone Reactor	Feed P	500 mg/L COD			-0.065	69	409	1347	872
13	21.08.2014	Control Reactor	Feed 1	500 mg/L COD	2	11.6	-0.095	60	384	1047	786
		Glucose Reactor	Feed G	500 mg/L COD			-0.081	56	414	881	765
		Peptone Reactor	Feed P	500 mg/L COD			-0.062	74	303	1418	901
14	26.08.2014	Control Reactor	Feed 1	500 mg/L COD	4	11.6	-0.090	50	396	1052	784
		Glucose Reactor	Feed G	500 mg/L COD			-0.099	54	421	855	745
		Peptone Reactor	Feed P	500 mg/L COD			-0.060	71	396	1142	851
15	28.08.2014	Control Reactor	Feed 1	500 mg/L COD	3	11.6	-0.076	66	393	885	748
		Glucose Reactor	Feed G	500 mg/L COD			-0.092	50	388	869	775
		Peptone Reactor	Feed P	500 mg/L COD			-0.056	75	384	1140	821

SC: Surface Charge
Hydro: Hydrophobicity

Table 4.4. Properties of respirometric tests shown in chronological order (continued).

Test No:	Date	Reactor Name	Feed	THEORETICAL VALUES			ANALYTICAL VALUES				
				Feed Concentration	Ag Concentration (mg/L)	ATU (mg/L)	Initial SC (meqv/g SS)	Initial Hydro. (%)	Initial COD (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
16	02.09.2014	Control Reactor	Feed 1	500 mg/L COD	5	11.6	-0.075	54	408	1062	818
		Glucose Reactor	Feed G	500 mg/L COD			-0.089	42	428	910	800
		Peptone Reactor	Feed P	500 mg/L COD			-0.052	76	355	1366	920
17	04.09.2014	Control Reactor	Feed 1	500 mg/L COD	2	11.6	-0.068	58	363	1181	894
		Glucose Reactor	Feed G	500 mg/L COD			-0.087	56	357	914	806
		Peptone Reactor	Feed P	500 mg/L COD			-0.069	68	385	1220	907
18	20.10.2014	Peptone Reactor	Feed P	500 mg/L COD	3 and 5	0	not measured	not measured	430	not measured	not measured
			Feed G	500 mg/L COD	3 and 5				457		
19	27.10.2014	Glucose Reactor	Feed G	500 mg/L COD	3 and 5	0	not measured	not measured	366	not measured	not measured
			Feed P	500 mg/L COD	3 and 5				491		
20	10.11.2014	Reactor 3	Feed 3	50 mg/L NH_4-N	0.25, 0.75 and 1	0	not measured	not measured	-	not measured	not measured

SC: Surface Charge
Hydro: Hydrophobicity

Additionally, the chronological order of respirometric tests during the operation of CR, RG and RP is shown in Figure 4.13., Figure 4.14. and Figure 4.15, respectively. These figures also show the respective loading rates (F/M) and specific substrate removal rates (q). In CR, RG and RP the average F/M ratio was found as 0.17±0.03, 0.14±0.04 and 0.14±0.02 mg COD/mg MLVSS.day, respectively. Also, the average q in these sludges was found as 0.15±0.03, 0.13±0.04 and 0.12±0.02 mg COD/mg MLVSS.day, respectively.

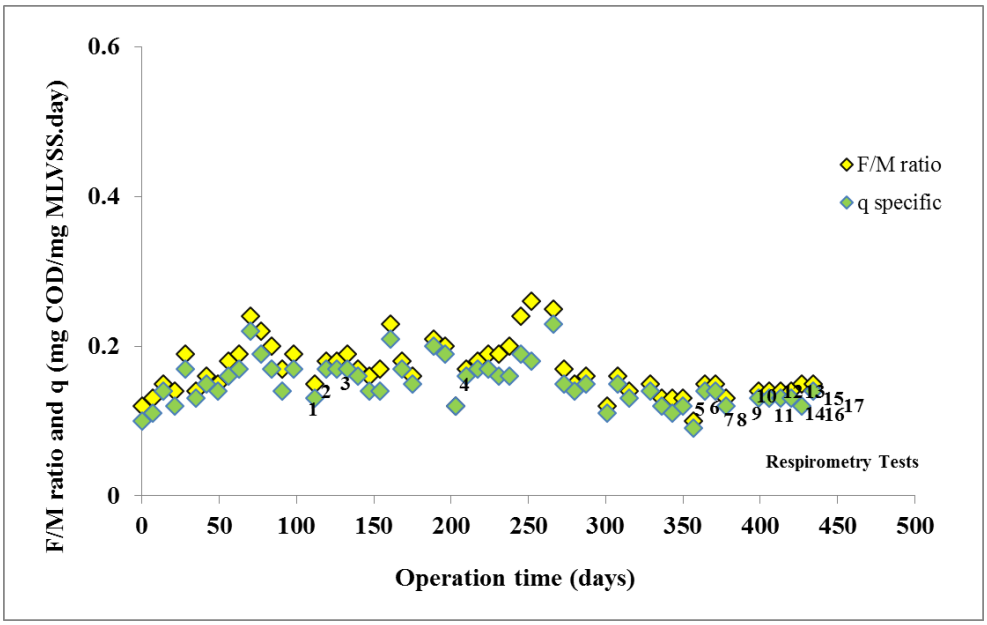

Figure 4.13. Respirometry Tests in CR Operation.

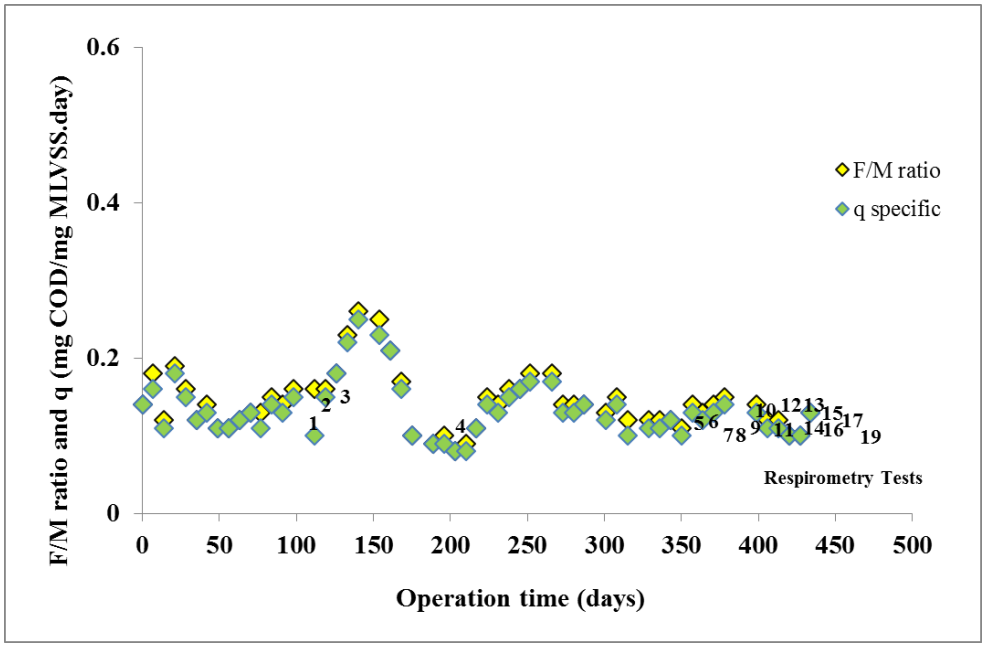

Figure 4.14. Respirometry Tests in RG Operation.

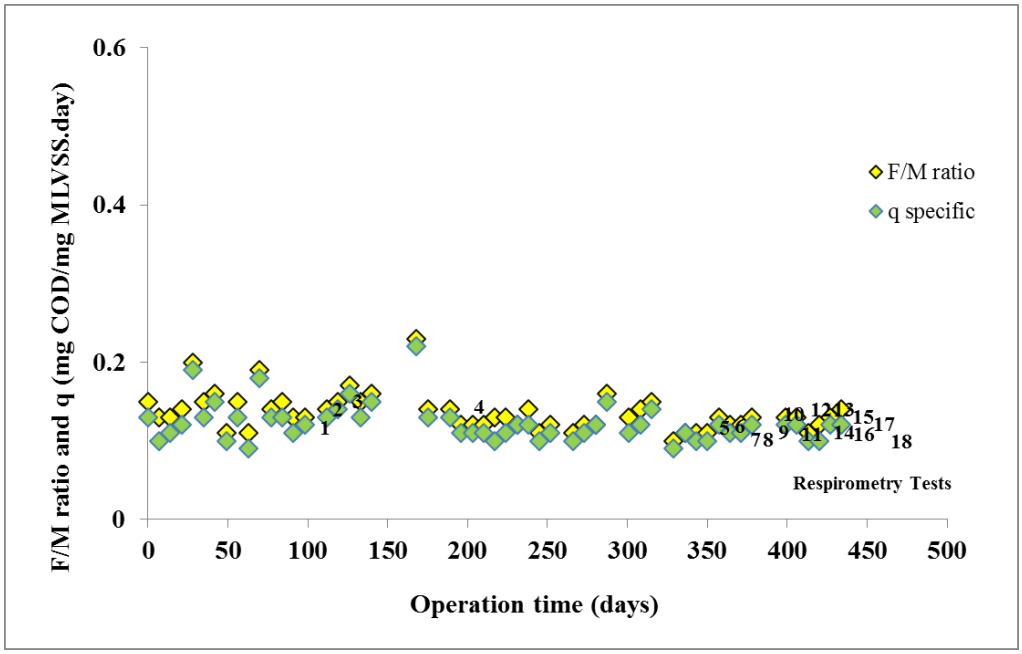

Figure 4.15. Respirometry Tests in RP Operation.

4.2.1. Determination of Organic Carbon Removal and Nitrification

Respirometry tests were carried out in order to observe carbonaceous oxygen uptake (C-O_2), nitrogenous oxygen uptake (N-O_2), carbonaceous carbon dioxide production (C-CO_2) and nitrogenous carbon dioxide production (N-CO_2) in different sludges. The details of procedures were given in "Materials and Methods".

4.2.1.1. Results of Control Reactor (CR): In **Respirometric Test 9** ATU was used as a nitrification inhibitor. The cumulative oxygen uptake in terms of total oxygen uptake (T-O_2), carbonaceous oxygen uptake (C-O_2) and nitrogenous oxygen uptake (N-O_2) is shown in Figure 4.16. T-O_2 and C-O_2 show the total and carbonaceous oxygen uptake, respectively. In calculation of these values, endogenous respiration was substracted. According to Figure 4.16., the heterotrophic activity in this sludge was greater than autotrophic activity. Most of oxygen was consumed in organic carbon removal rather than nitrification. The oxygen uptake due to organic carbon removal (T-O_2) is seen as 35.36 mg O_2 while nitrification is about 11 mg O_2 (N-O_2). Additionally, Figure 4.17. shows the cumulative carbon dioxide production in terms of total carbon dioxide production (T-CO_2), carbonaceous carbon dioxide production (C-CO_2) and nitrogenous carbon dioxide production (N-CO_2). According

to these figures, the percentage of N-O_2 in T-O_2 and the percentage of N-CO_2 in T-CO_2 were calculated as 30 % and 35 %, respectively.

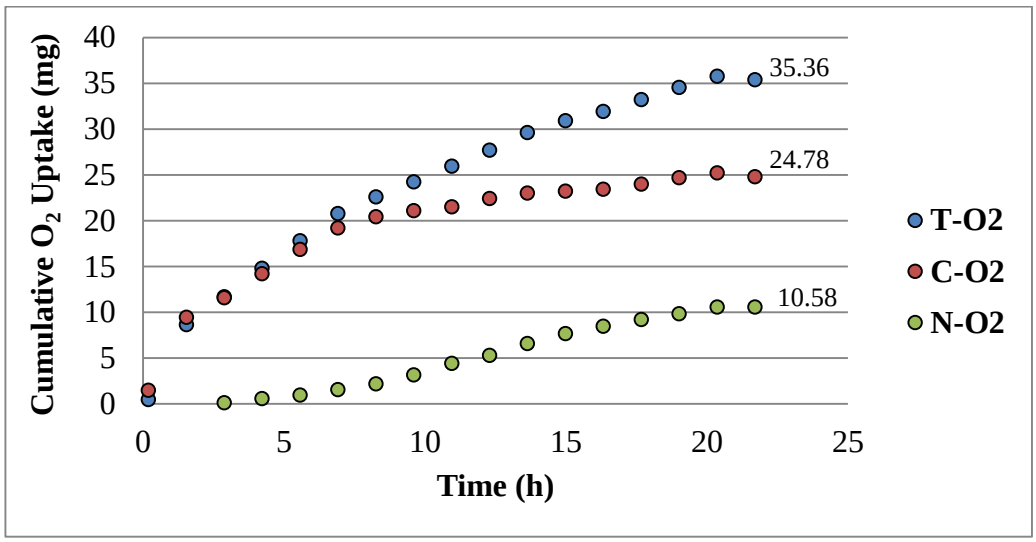

Figure 4.16. T-O_2, C-O_2 and N-O_2 results in Respirometric Test 9 (CR-05.08.2014).

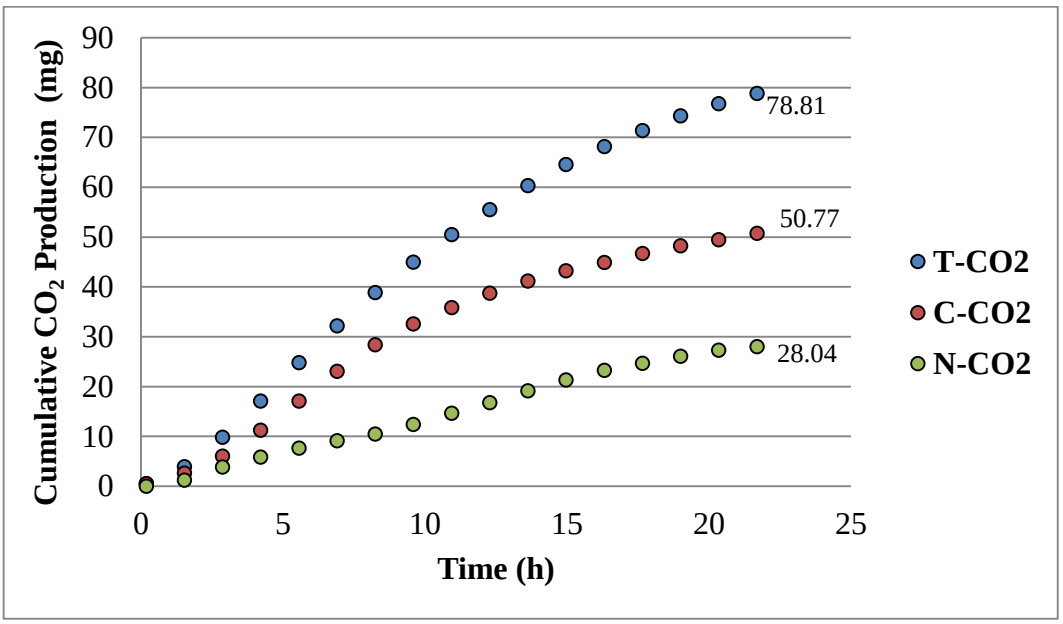

Figure 4.17. T-CO_2, C-CO_2 and N-CO_2 results in Respirometric Test 9 (CR-05.08.2014).

In respirometric **Tests from 9 to 17,** ATU was used in order to determine C-O_2 and N-O_2. According to results, nitrogenous oxygen uptake (N-O_2 due to nitrification) was found as approximately 11-12 mg O_2 in the Control Reactor. In **Respirometric Tests 12 and 15,**

$N\text{-}O_2$ values were calculated as 7 mg O_2 and 8 mg O_2, respectively. Figure 4.18. and Figure 4.19. show the $T\text{-}O_2$, $C\text{-}O_2$ and $N\text{-}O_2$ values. The percentages of $N\text{-}O_2$ in $T\text{-}O_2$ in **Test 12 and 15** were calculated as 32 % and 28 %, respectively. These results show that organic carbon removal was dominant in these tests.

In addition, in **Test 12,** $T\text{-}O_2$ was found as 24 mg which is lower than in other tests. Figure 4.18. indicates the rapid consumption of organic carbon. As a result, $C\text{-}O_2$ reached a constant value with time, while $N\text{-}O_2$ was increasing. As seen in Figure 4.18., after 8th hour, $N\text{-}O_2$ increased due to consumption of organic matter. The negative results which have no physical meaning were corrected as zero.

In Figure 4.19., total oxygen uptake was seen as 32.08 mg. This value does not match with Figure 4.30, because endogenous respiration was substracted from total oxygen uptake. Also in other figures, endogenous respiration was substracted. Therefore, in these figures $T\text{-}O_2$ values are lower than in figures illustrating raw data.

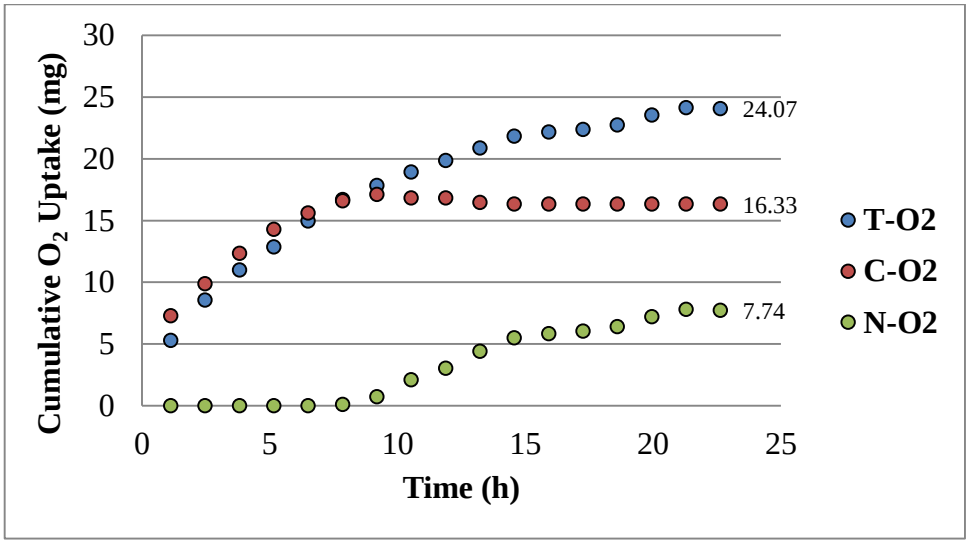

Figure 4.18. $T\text{-}O_2$, $C\text{-}O_2$ and $N\text{-}O_2$ results in Respirometric Test 12 (CR-19.08.2014).

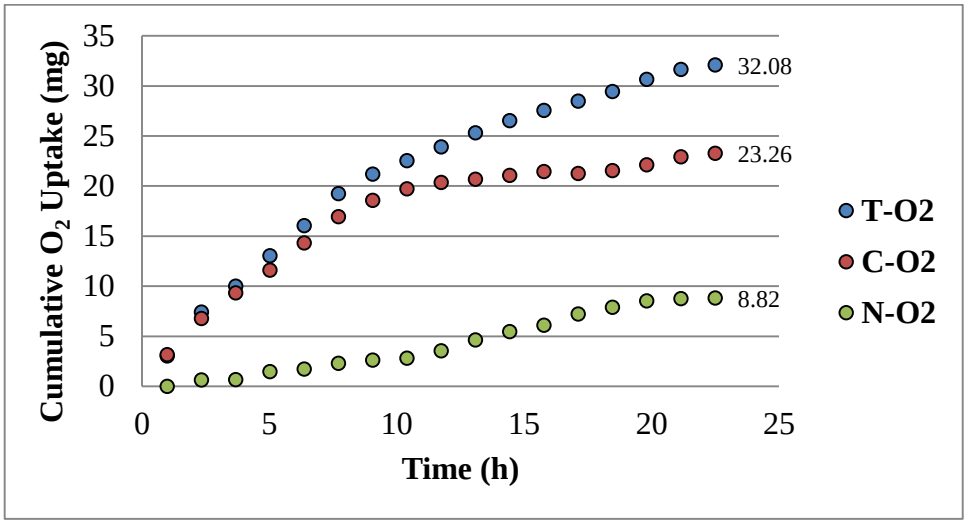

Figure 4.19. T-O_2, C-O_2 and N-O_2 results in Respirometric Test 15 (CR-28.08.2014).

Respirometric and analytical results belonging to the respirometric tests were also examined. By using the analytical measurements and measured C-O_2 value, Y_h was calculated. As an example, analytical measurements in **Test 11** showed that COD removal in ATU-containing chamber (indicating organic carbon removal only) was 391 mg/L COD and measured C-O_2 was 21.62 mg O_2. According to this data, Y_h was calculated as follows:

391 mg COD / L x (1 – Y_h) x 0.1 L sample volume (4.5)
= 21.62 mg O_2 (measured C-O_2 value)
Y_h = 0.45 mg COD/mg COD

Average Y_h for CR sludge was found as 0.5±0.09 g cell COD/g substrate COD. This value is slightly lower than the theoretical assumption which is 0.6 g cell COD/g substrate COD (Rittman and McCarty, 2001).

Overall, respirometric tests with ATU addition showed that the nitrification inhibitor had a small effect on CR sludge. There was a relatively small difference between T-O_2 and C-O_2, indicating that the oxygen uptake due to nitrification was already small during the test period. The reason is that heterotrophic activity was more dominant than nitrifying activity in this sludge that is operated at the C/N ratio of 10.

4.2.1.2. Results of Glucose Reactor (RG): In **Respirometric Test 10** ATU was used as a nitrification inhibitor. T-O_2, C-O_2 and N-O_2 values are shown in Figure 4.20. According to the figure, N-O_2 value at the end of the test was 7.58 mg which is close to the results of **Respirometric Test 13 and 16** (7.59 mg and 7.63 mg O_2, respectively). Moreover, Figure 4.21. shows the cumulative carbon dioxide production in terms of total carbon dioxide production (T-CO_2), carbonaceous carbon dioxide production (C-CO_2) and nitrogenous carbon dioxide production (N-CO_2). According to these figures, the percentage of N-O_2 in T-O_2 and the percentage of N-CO_2 in T-CO_2 were calculated as 24.2 % and 22.4 %, respectively. These values are lower compared to the CR sludge indicating that RG sludge had slightly higher heterotrophic activity.

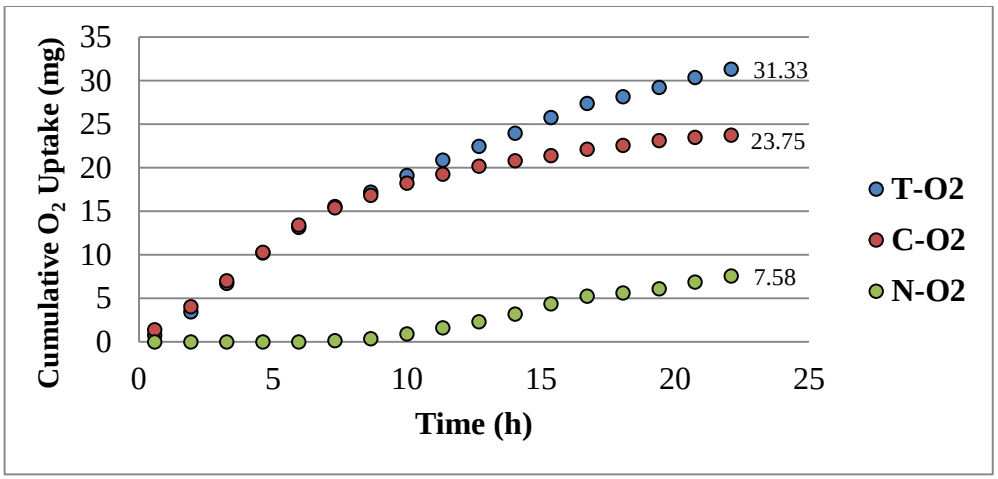

Figure 4.20. T-O_2, C-O_2 and N-O_2 results in Respirometric Test 10 (RG-07.08.2014).

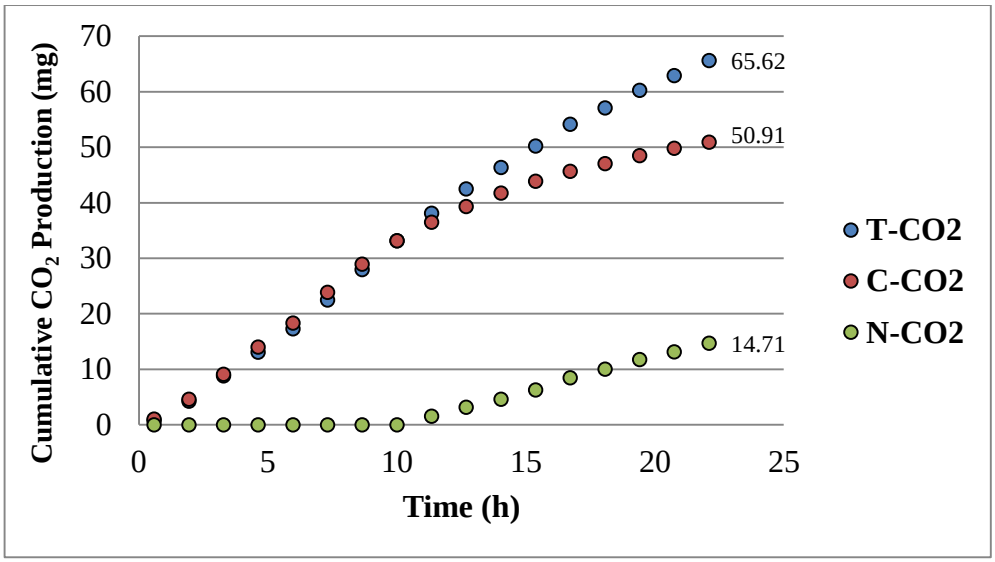

Figure 4.21. T-CO_2, C-CO_2 and N-CO_2 results in Respirometric Test 10 (RG-07.08.2014).

It was seen that T-O_2 and C-O_2 values were close to each other until the 10th hour. Then, T-O_2 and C-O_2 separated from each other and N-O_2 started to increase. This indicates that organic carbon removal dominates in the sludge until 10th hour and then nitrification starts because of the depletion of organic matter.

Respirometric and analytical results belonging to the respirometric tests were also examined. By using the analytical measurements and measured C-O_2 value, Y_h was calculated. As an example, analytical measurements in **Test 16** showed that COD removal in ATU-containing chamber (indicating organic carbon removal only) was 414 mg/L COD and measured C-O_2 was 19.21 mg O_2. According to this data, Y_h was calculated as follows:

414 mg COD / L x (1 – Y_h) x 0.1 L sample volume (4.6)
= 19.21 mg O_2 (measured C-O_2 value)
Y_h = 0.54 mg COD/mg COD

Average Y_h for RG sludge was found as 0.54±0.1 g cell COD/g substrate COD. This value is slightly lower than the theoretical assumption which is 0.6 g cell COD/g substrate COD (Rittman and McCarty, 2001).

Overall, respirometric tests with ATU addition showed that the nitrification inhibitor had a small effect on the total respiration of RG sludge. According to the results, this sludge removed primarily organic carbon. This was seen because there was a small difference between T-O_2 and C-O_2, indicating that the oxygen uptake due to nitrification was small, similar to CR sludge. But in the RG sludge the nitrifying activity was slightly lower in terms of N-O_2 values compared to CR sludge. Moreover, results indicated that total oxygen uptake in this sludge was lower than in CR.

4.2.1.3. Results of Peptone Reactor (RP): **Respirometric Test 11** was carried out with ATU as a nitrification inhibitor. T-O_2, C-O_2 and N-O_2 values are shown in Figure 4.22. According to the figure, N-O_2 value at the end of the test is 15.76 mg which is higher compared to CR and RG reactors. Moreover, Figure 4.23. shows the cumulative carbon dioxide production in terms of total carbon dioxide production (T-CO_2), carbonaceous carbon dioxide production (C-CO_2) and nitrogenous carbon dioxide production (N-CO_2). According to these

figures, the percentage of N-O_2 in T-O_2 and the percentage of N-CO_2 in T-CO_2 were calculated as 38.4 % and 32.7 %, respectively.

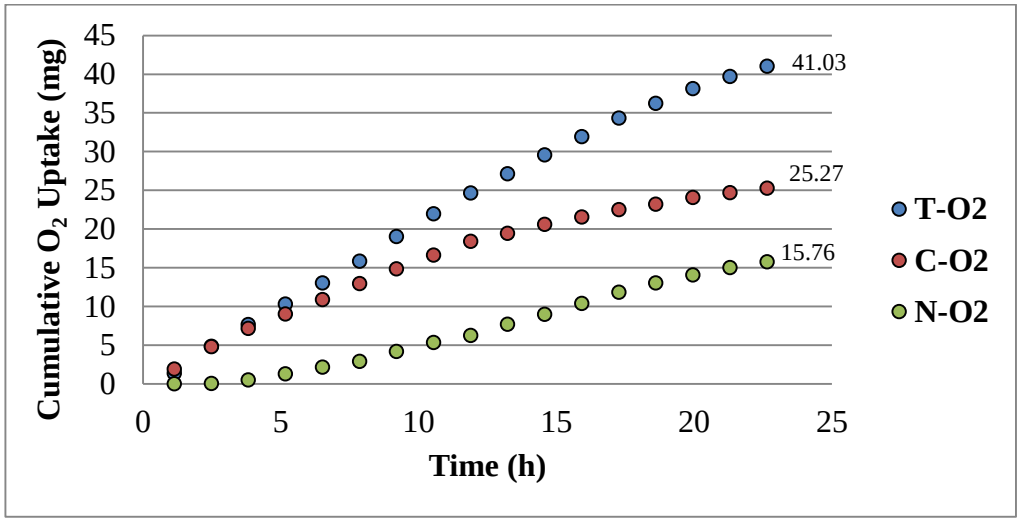

Figure 4.22. T-O_2, C-O_2 and N-O_2 results in Respirometric Test 11 (RP-12.08.2014).

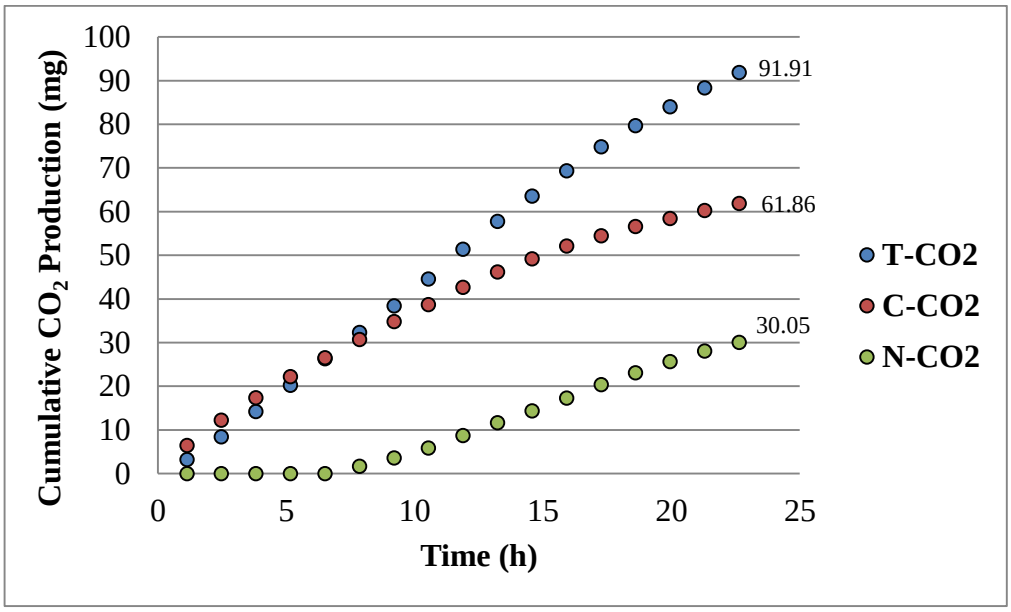

Figure 4.23. T-CO_2, C-CO_2 and N-CO_2 results in Respirometric Test 11 (RP-12.08.2014).

According to Figure 4.22, T-O_2 and C-O_2 values were close to each other until 6th hour. Then, T-O_2 and C-O_2 separated from each other since N-O_2 started to increase. This indicates an earlier start of nitrification compared with the RG reactor. In the RP sludge nitrifiers were

more active compared to the other reactors. Also, the high nitrogenous oxygen uptakes support this idea.

In **Respirometric Test 13,** it was seen that C-O_2 and N-O_2 values were close to each other at the end of the test. According to Figure 4.24., nitrogenous oxygen uptake starts to increase after 7th hour and reaches 15.88 mg which is close to C-O_2 value (18.04 mg). This indicates that approximately 50% of total oxygen uptake consists of nitrification activity. Other tests showed that nitrogenous oxygen uptake (N-O_2) was about 15-17 mg O_2 in RP sludge.

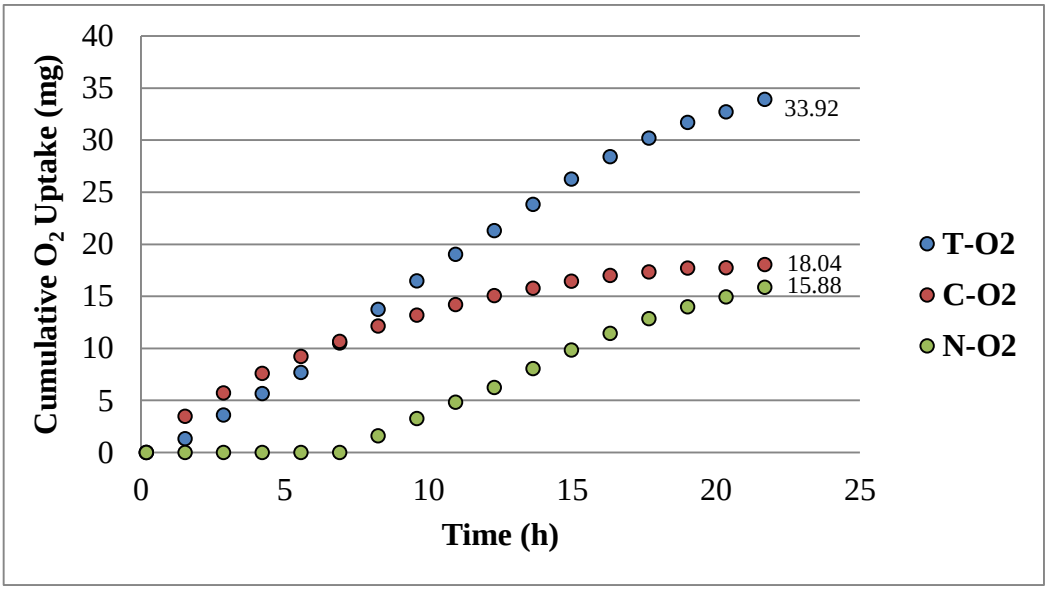

Figure 4.24. T-O_2, C-O_2 and N-O_2 results in Respirometric Test 13 (RP-21.08.2014).

Respirometric and analytical results belonging to the respirometric tests were also examined. By using the analytical measurements and measured C-O_2 value, Y_h was calculated. As an example, analytical measurements in **Test 17** showed that COD removal in ATU-containing chamber (indicating organic carbon removal only) was 323 mg/L COD and measured C-O_2 was 14.76 mg O_2. According to this data, Y_h was calculated as follows:

323 mg COD / L x (1 – Y_h) x 0.1 L sample volume (4.7)

= 14.76 mg O_2 (measured C-O_2 value)

Y_h = 0.54 mg COD/mg COD

Average Y_h for RP sludge was found as 0.51±0.15 g cell COD/g substrate COD. This value is slightly lower than the theoretical assumption which is 0.6 g cell COD/g substrate COD (Rittman and McCarty, 2001).

Overall, results showed that the nitrification inhibitor ATU affected RP reactor more than other reactors. According to results, T-O_2 and C-O_2 were relatively different from each other, indicating that the oxygen uptake due to nitrification was not as small in this sludge as in CR and RG sludges. It can be said that RP sludge had a higher nitrification activity than CR and RG reactors.

4.2.2. Determination of the Inhibitory Effect of Ag on Activated Sludge

In this part of the study, the aim was to determine the effect of Ag on different types of activated sludges that were operated at the same C/N ratio. Since these reactors were fed with different feeds, the inhibitory effect of Ag was expected to differ. The inhibitory effect of Ag was measured by respirometric tests. In these tests, Ag concentration ranged from 2 to 5 mg/L.

4.2.2.1. Results of Control Reactor (CR): According to a previous study, 1 mg/L Ag had no effect on the performance of this sludge (Ayyıldız, 2013). So, the minimum concentration used in the respirometric tests was selected as 2 mg/L Ag. **Respirometric Tests 9, 13 and 17** were carried out at this concentration. Figure 4.25. shows that in **Test 13,** 2 mg/L Ag had no effect on CR sludge, as seen from cumulative oxygen uptakes that were close to each other.

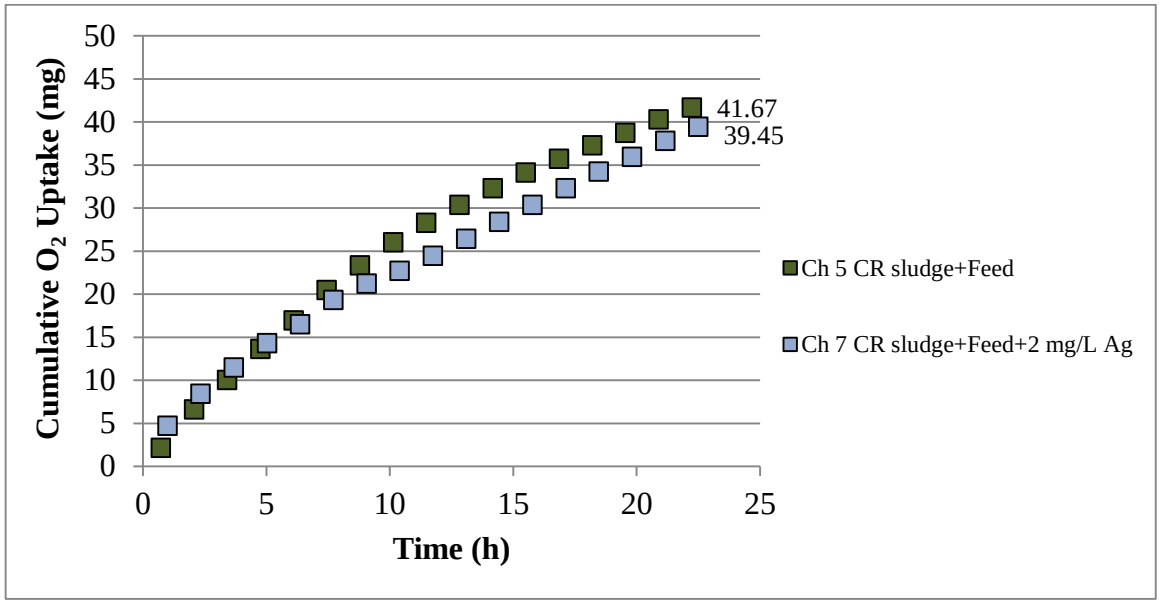

Figure 4.25. Results in Respirometric Test 13 (CR-21.08.2014).

Test 17 showed the same trend as **Test 13.** Figure 4.26. shows the cumulative oxygen uptakes of control and metal-containing chambers. As seen in this figure that were very close to each other. Additionally, Figure 4.27. shows the carbon dioxide productions. This figure also indicates that 2 mg/L Ag did not affect the performance of this sludge.

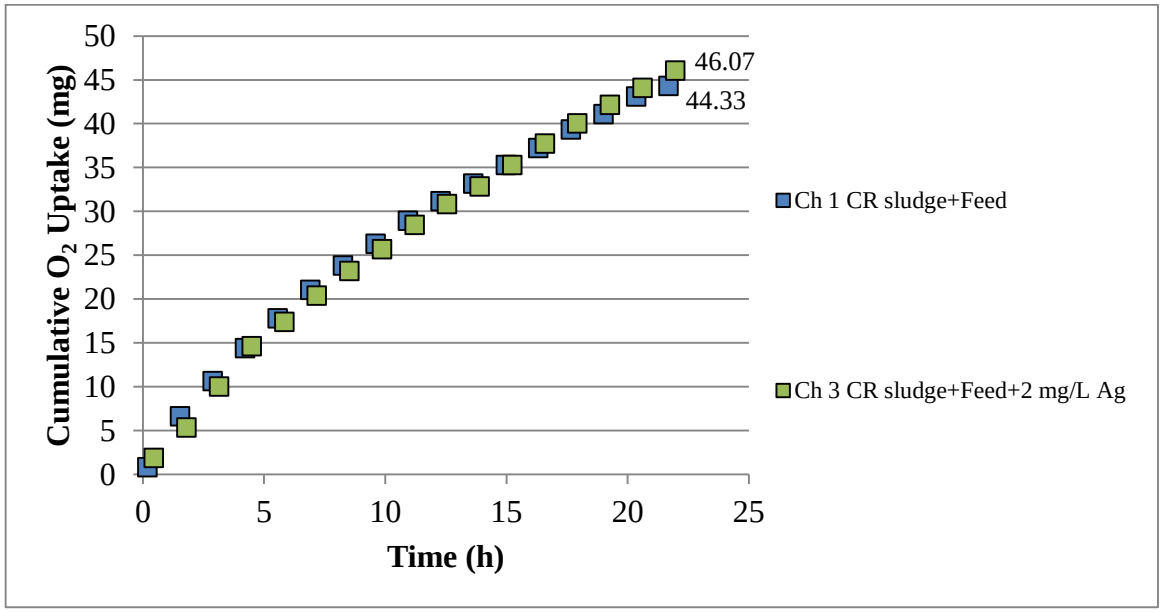

Figure 4.26. Results in Respirometric Test 17 (CR-04.09.2014).

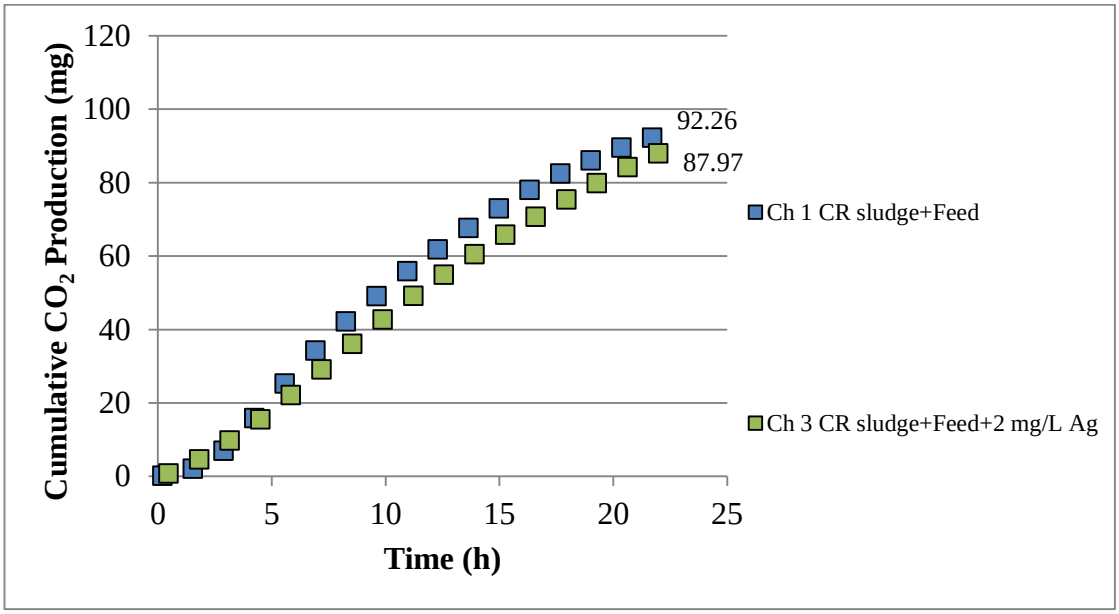

Figure 4.27. Results in Respirometric Test 17 (CR-04.09.2014).

Respirometric Tests 7, 10 and 15 were carried out at 3 mg/L Ag concentration. Results of the **Test 7** are shown in Figure 4.28. This figure shows that 3 mg/L Ag affected the sludge. Besides cumulative values, also O_2 uptake rates were analyzed. As shown in Figure 4.29., the sludge fed with 3 mg/L Ag had very low O_2 uptake rates.

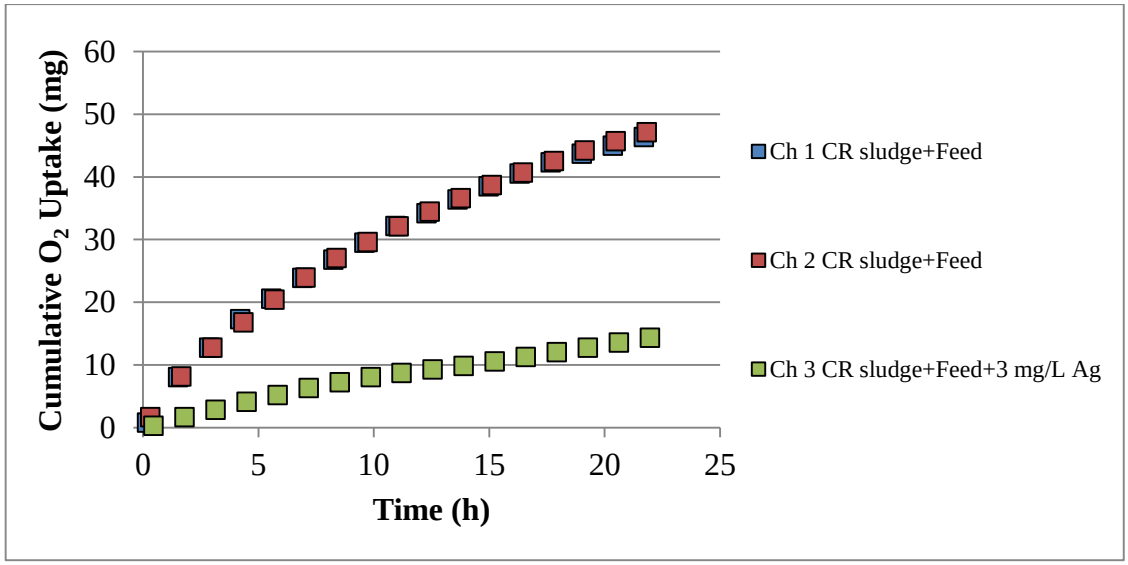

Figure 4.28. Results in Respirometric Test 7 (CR-10.07.2014).

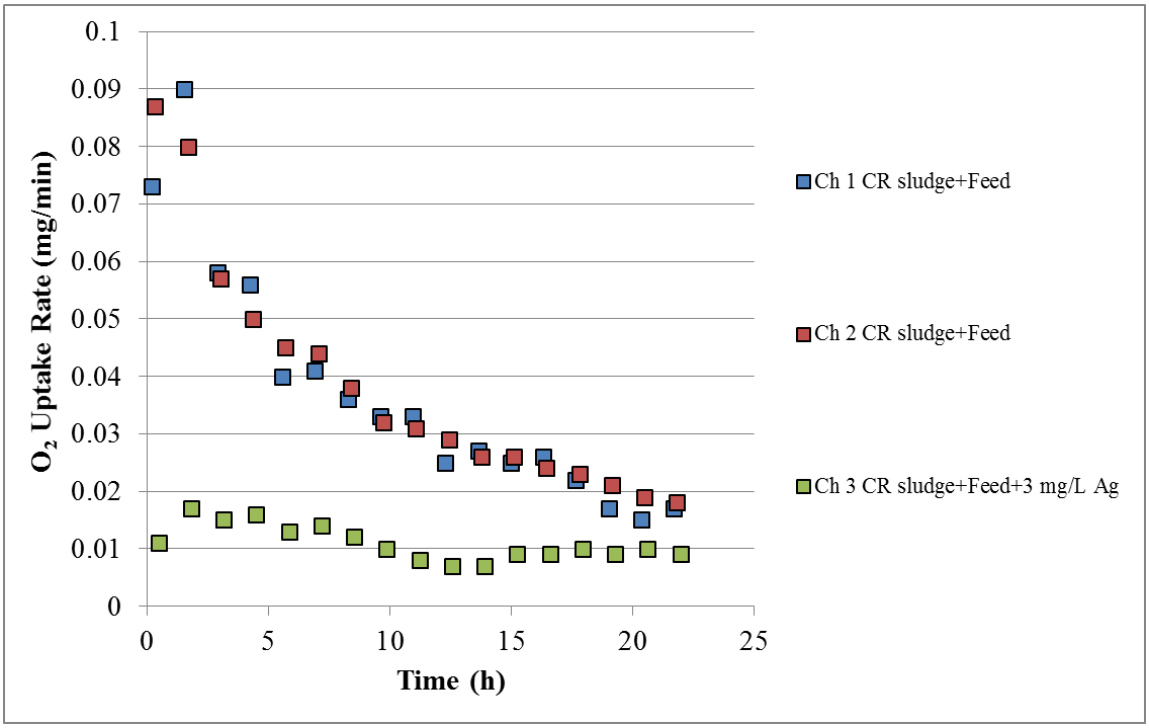

Figure 4.29. Oxygen uptake rates in Respirometric Test 7 (CR-10.07.2014).

On the other hand, as shown in Figure 4.30., in **Respirometric Test 15,** opposite results were observed. In this test, 3 mg/L Ag had no effect on CR sludge. Total oxygen uptake values of control sludge and metal-containing sludge were very close to each other.

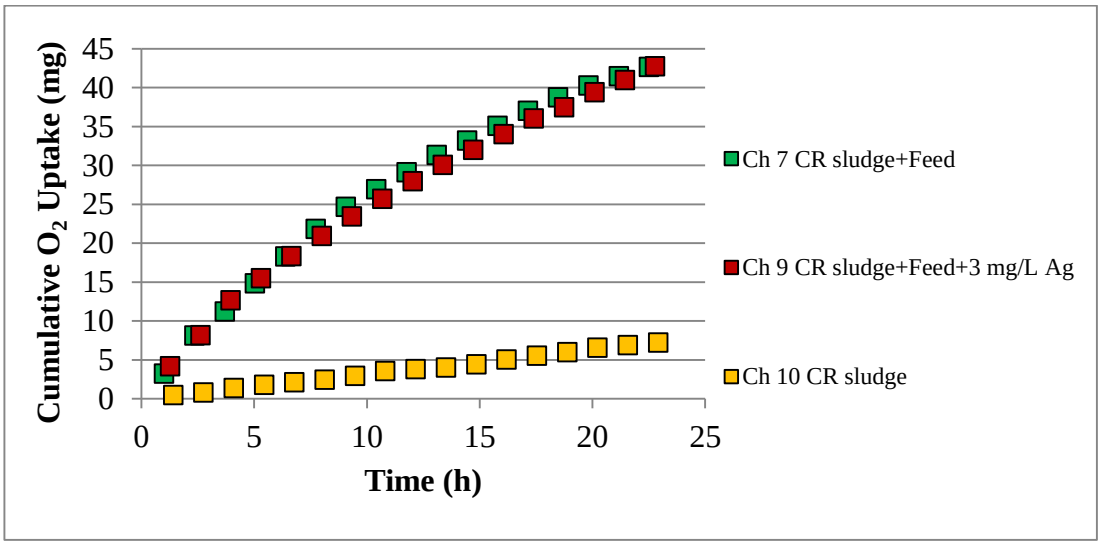

Figure 4.30. Results in Respirometric Test 15 (CR-28.08.2014).

Respirometric Tests 6, 11 and 14 were carried out at 4 mg/L Ag concentration. The results of these three tests were close to each other. Figure 4. 31. shows that 4 mg/L Ag had

a very inhibitory effect on this sludge. Total oxygen uptake values were very close to endogenous respiration value which indicates the total inhibition of sludge respiration.

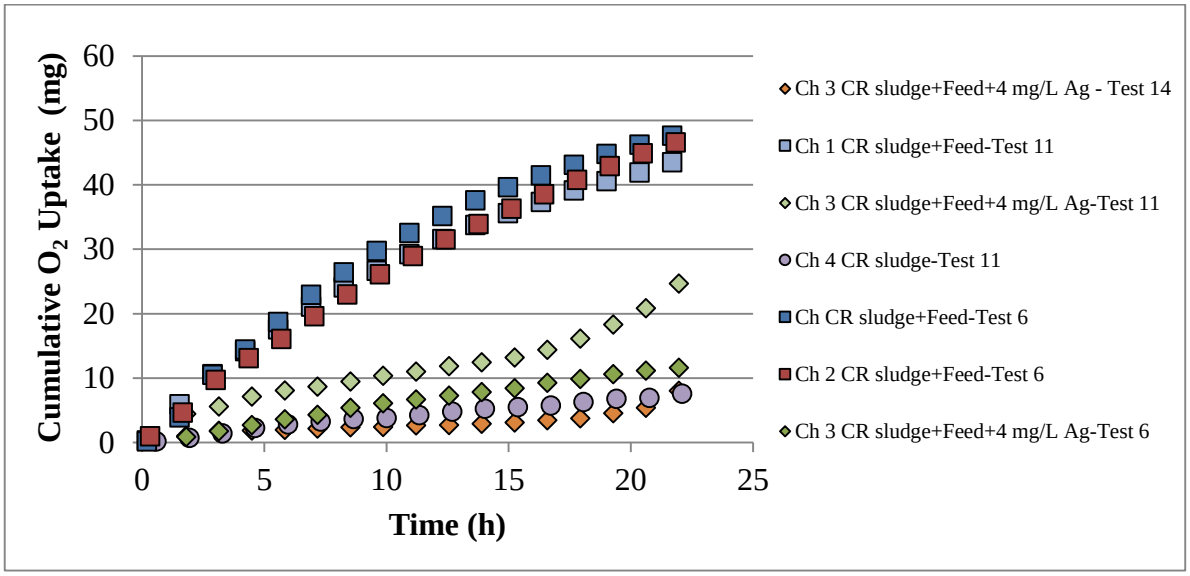

Figure 4. 31. Cumulative oxygen uptakes in Respirometric Test 6, 11 and 14 (CR).

In **Respirometric Test 11,** total oxygen uptake of metal-containing chamber started to increase after 20th hour. The reason of that was probably due to an error in the measurement of oxygen uptake rates during the test period.

Respirometric Tests 5, 12 and 16 were carried out at 5 mg/L Ag concentration. As shown in Figure 4. 32., results of these three tests were close to each other; 5 mg/L Ag inhibited this sludge totally. Total oxygen uptakes were even lower than endogenous respiration.

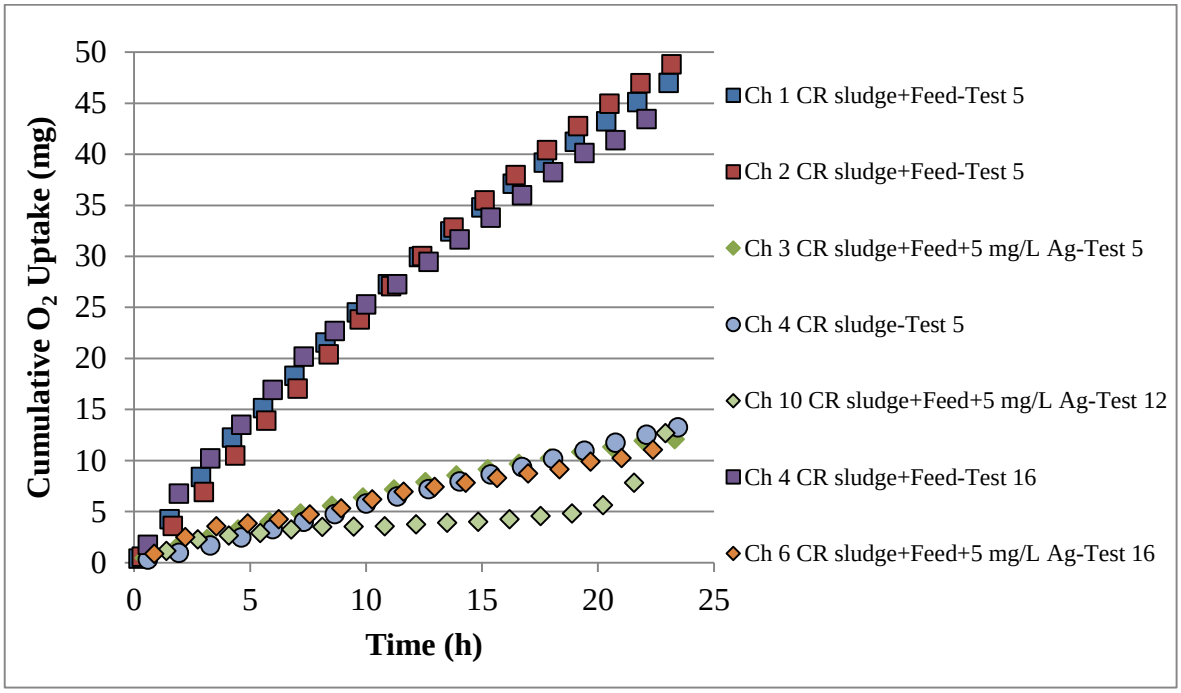

Figure 4. 32. Results in Respirometric Test 5, 12 and 16 (CR).

Moreover, when carbon dioxide production of control and metal-containing chambers were analyzed, the same result was observed. Figure 4. 33. shows the carbon dioxide production in **Tests 5, 12 and 16.** This figure indicates that 5 mg/L Ag addition highly affected the performance of CR sludge.

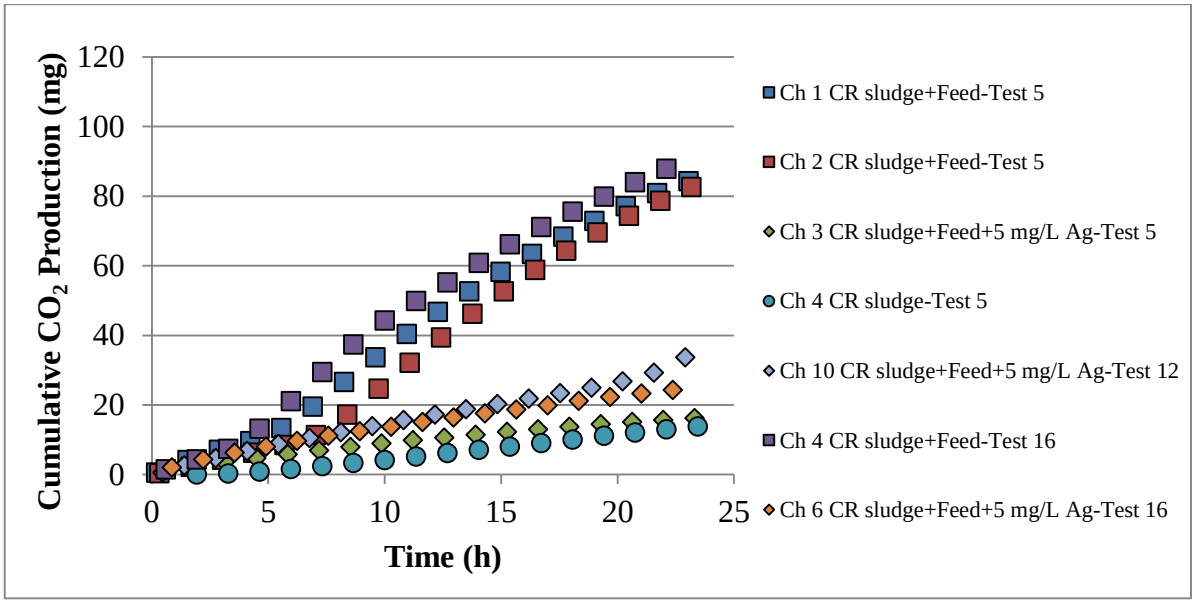

Figure 4. 33. Results in Respirometric Test 5, 12 and 16 (CR).

Overall, respirometry tests showed that 2 mg/L Ag had no effect on CR sludge. Moreover, 3, 4 and 5 mg/L Ag concentrations highly affected the performance of the sludge. The percent inhibition due to Ag addition is presented in Table 4.5. The percent inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Cumulative } O_2 \text{ (control) - Cumulative } O_2 \text{ (mg/L Ag)}}{\text{Cumulative } O_2 \text{ (control)}} \times 100 \qquad (4.8)$$

Similarly, the percent decrease in cumulative carbon dioxide production was calculated in the same way. Results show the average of respirometric tests. Results showed that carbon dioxide production was also highly affected by 3, 4 and 5 mg/L Ag concentrations.

Table 4.5. Inhibitory effect of Ag on CR sludge.

Ag Concentration (mg/L)	% Inhibition in T-O_2	% Inhibition in T-CO_2
2	2	6
3	85	78
4	74	70
5	77	73

4.2.2.2. Results of Glucose Reactor (RG): **Respirometric 13 and 17** were carried out at 2 mg/L Ag. Results of these tests were close to each other. Figure 4.34. shows that 2 mg/L Ag had no effect on RG sludge, as seen from total cumulative oxygen uptakes that were close to each other.

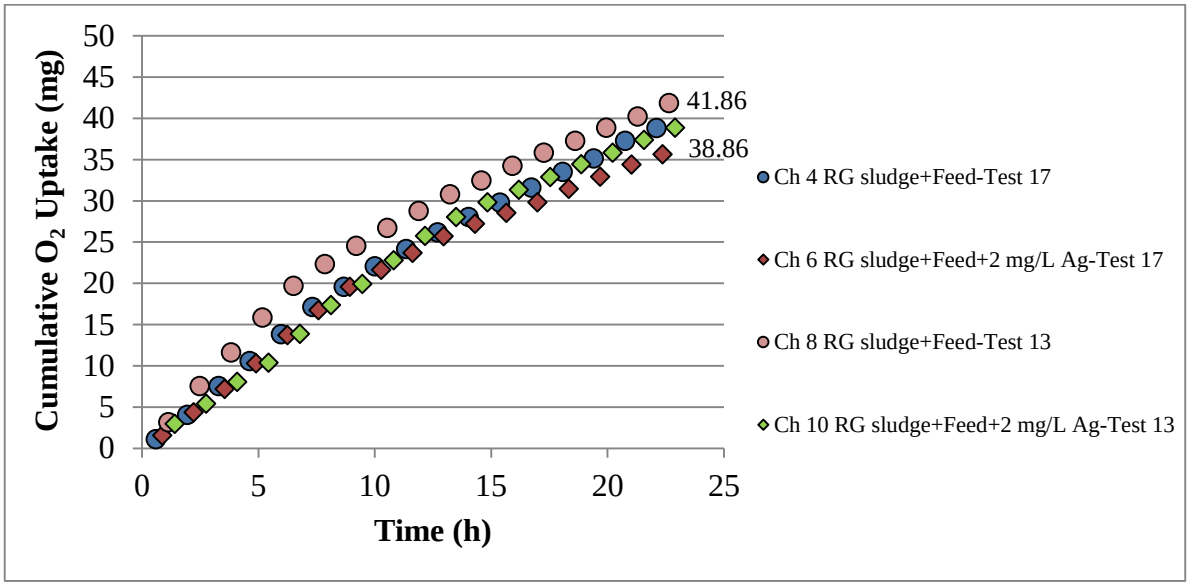

Figure 4.34. Results in Respirometric Test 13 and 17 (RG).

Respirometric Tests 7, 10 and 15 were carried out at 3 mg/L Ag. Results of the **Test 7** are shown in Figure 4.35. This figure shows that 3 mg/L Ag affected RG sludge. Also, it can be seen that the total oxygen uptake in the metal-containing chamber was very close to endogenous respiration (RG sludge).

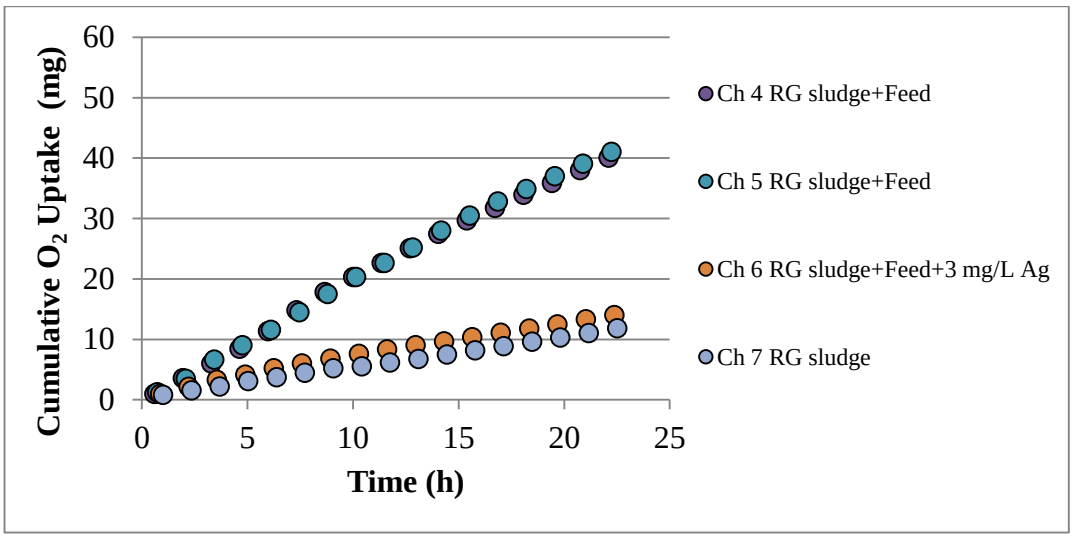

Figure 4.35. Results in Respirometric Test 7 (RG-10.07.2014).

On the other hand, in **Respirometric Test 10 and 15,** opposite results were observed. The results are shown in Figure 4.36. and Figure 4.37. It was seen that 3 mg/L Ag had no

effect on RG sludge. Total oxygen uptakes of control sludge and metal-containing sludge were very close to each other.

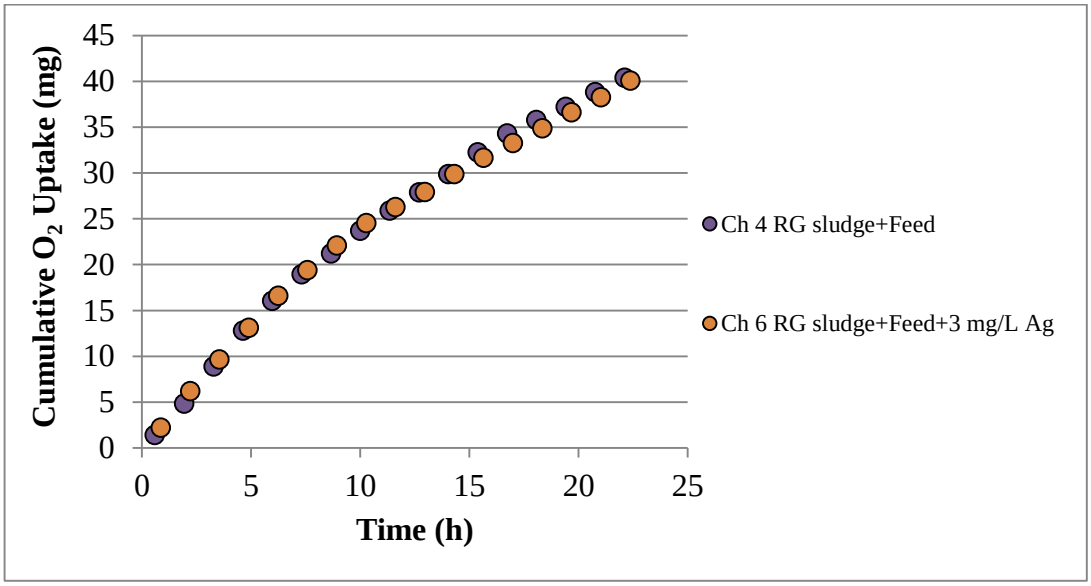

Figure 4.36. Results in Respirometric Test 10 (RG-07.08.2014).

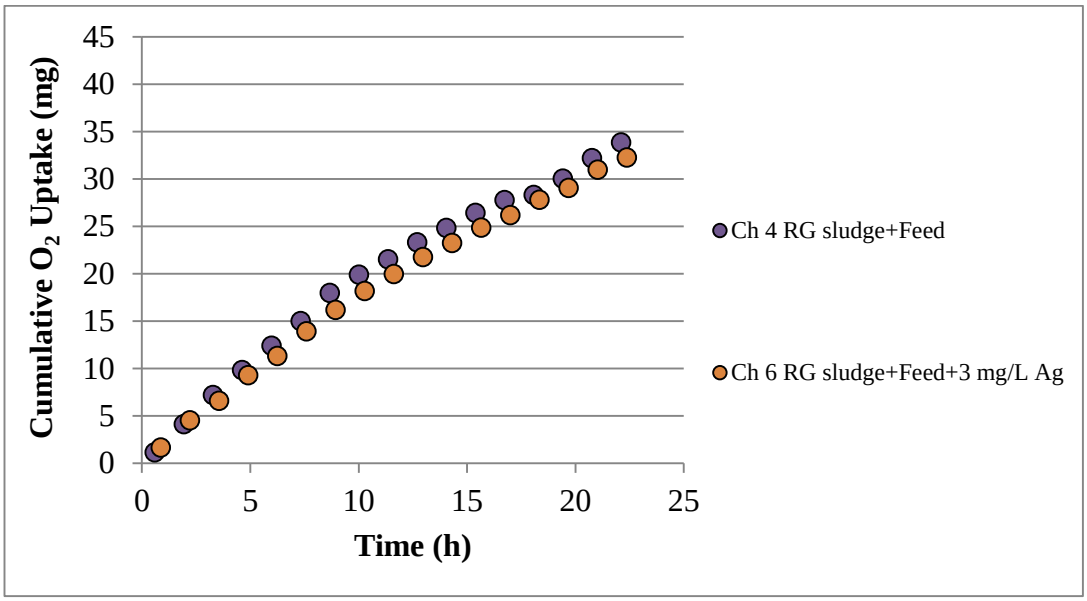

Figure 4.37. Results in Respirometric Test 15 (RG-28.08.2014).

Respirometric Tests 6, 11 and 14 were carried out at 4 mg/L Ag concentration. Results of **Test 6 and 14** were close to each other. Figure 4.38. shows that 4 mg/L Ag had an inhibitory effect on this sludge. Total oxygen uptakes in metal-containing chambers were

close to endogenous respiration (RG sludge) which indicates the inhibition of microorganisms.

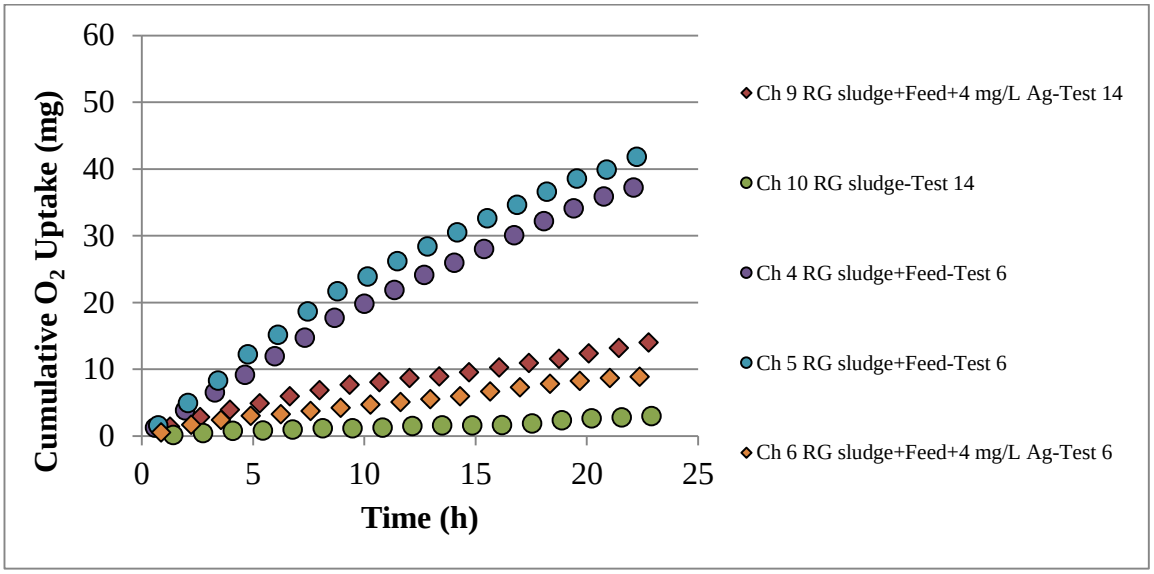

Figure 4.38. Results in Respirometric Test 6 and 14 (RG).

On the other hand, as shown in Figure 4.39., in **Respirometric Test 11,** opposite results were observed. According to this test, 4 mg/L Ag had a slight effect on this sludge. In this test, the initial MLVSS concentration was higher than in others. This might be a factor reducing the inhibitory effect of Ag. In order to show the effect of MLVSS concentration, Ag concentrations were normalized by MLVSS data. Table 4.6. indicates that at higher MLVSS concentrations the sludge was less affected by the addition of Ag.

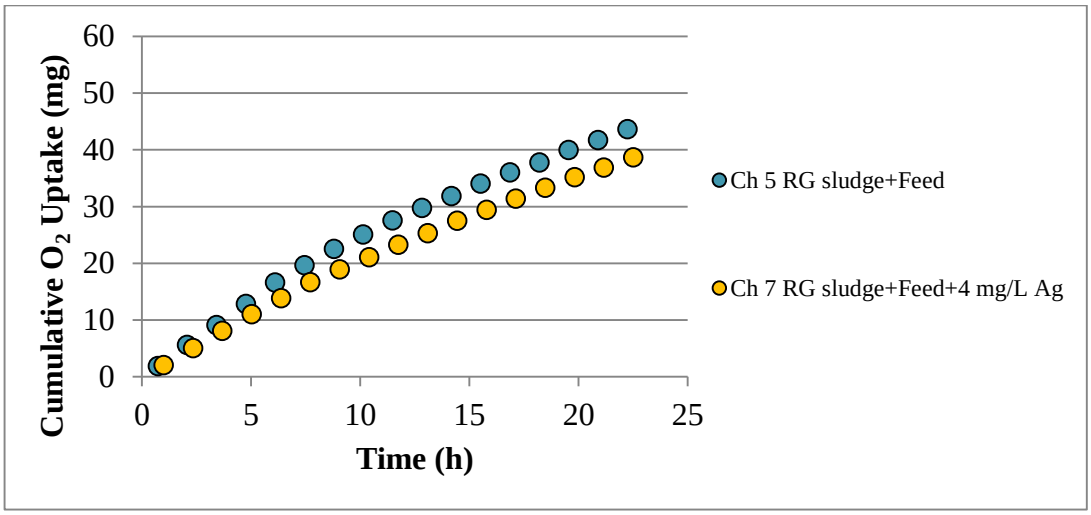

Figure 4.39. Results in Respirometric Test 11 (RG-12.08.2014).

Table 4.6. The relative Ag dosing per MLVSS (Tests 6, 11 and 14).

Test No	Ag (mg/L)	MLVSS (mg/L)	mg Ag / mg MLVSS	% Inhibition in T-O_2
6	4	688	0.0058	78
11	4	927	0.0043	11
14	4	745	0.0054	58

Also, when O_2 uptake rates were analyzed, as shown in Figure 4.40., it was seen that control and metal-containing chambers had very close values.

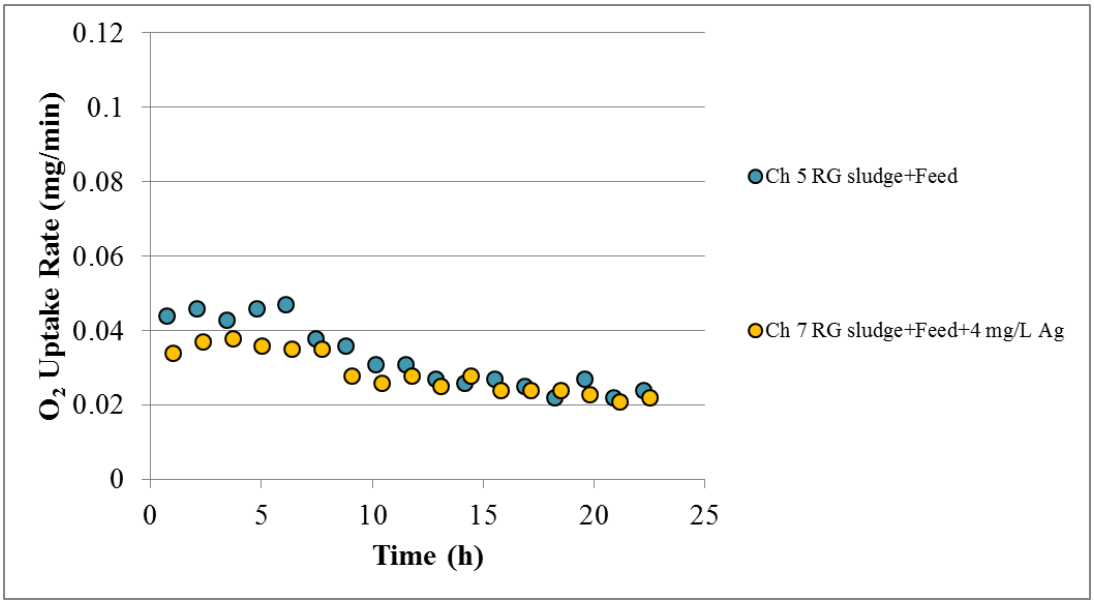

Figure 4.40. Results in Respirometric Test 11 (RG-12.08.2014).

Respirometric Tests 5, 12 and 16 were carried out at 5 mg/L Ag concentration. In **Respirometric Test 12,** it was seen that 5 mg/L Ag totally inhibited microorganisms. Results are shown in Figure 4.41. In addition, when O_2 uptake rates were analyzed, as shown in Figure 4.42., oxygen uptake rate was 0 mg/min in the metal-containing chamber which indicates total inhibition.

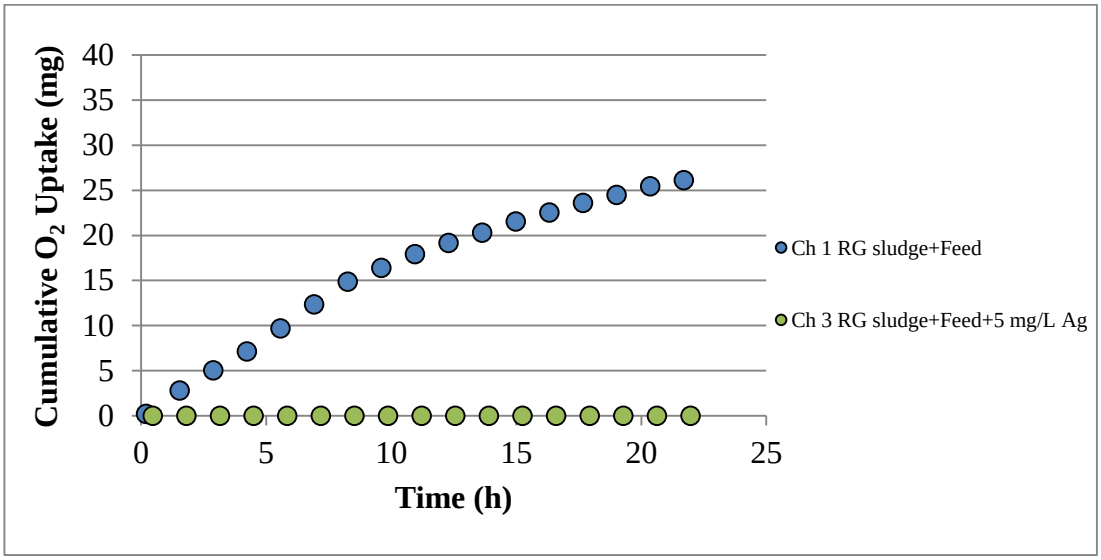

Figure 4.41. Results in Respirometric Test 12 (RG-19.08.2014).

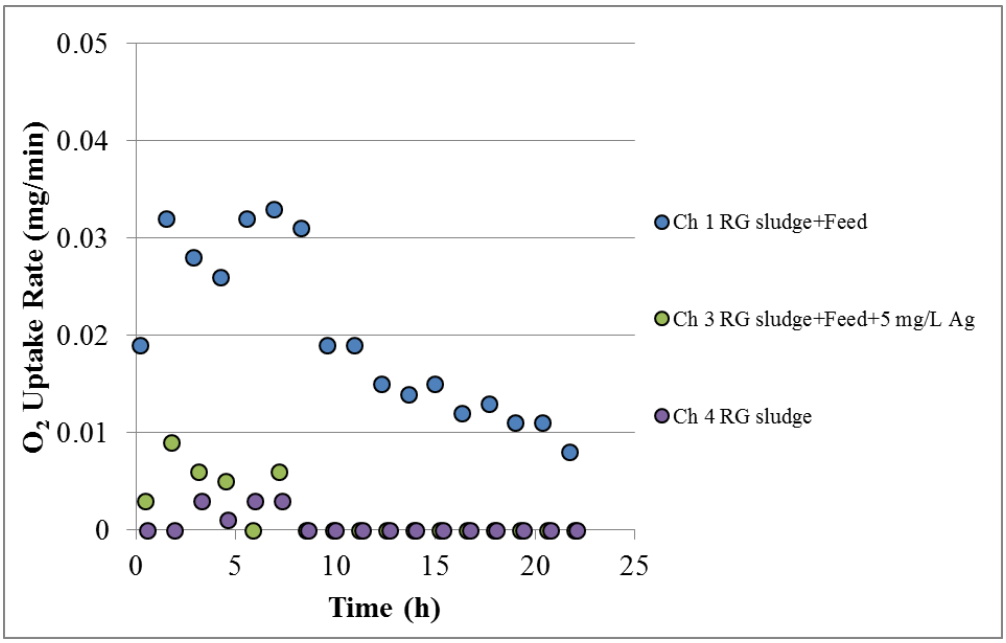

Figure 4.42. Results in Respirometric Test 12 (RG-19.08.2014).

Additionally, results of **Test 5 and 16** were close to each other. Figure 4.43. shows that 5 mg/L Ag inhibited this sludge. Figure 4.44. shows carbon dioxide productions in **Tests 5 and 16.** According to this figure, metal-containing chambers had a low CO_2 production compared to control chambers. This indicates that 5 mg/L Ag had a high inhibitory effect on this sludge.

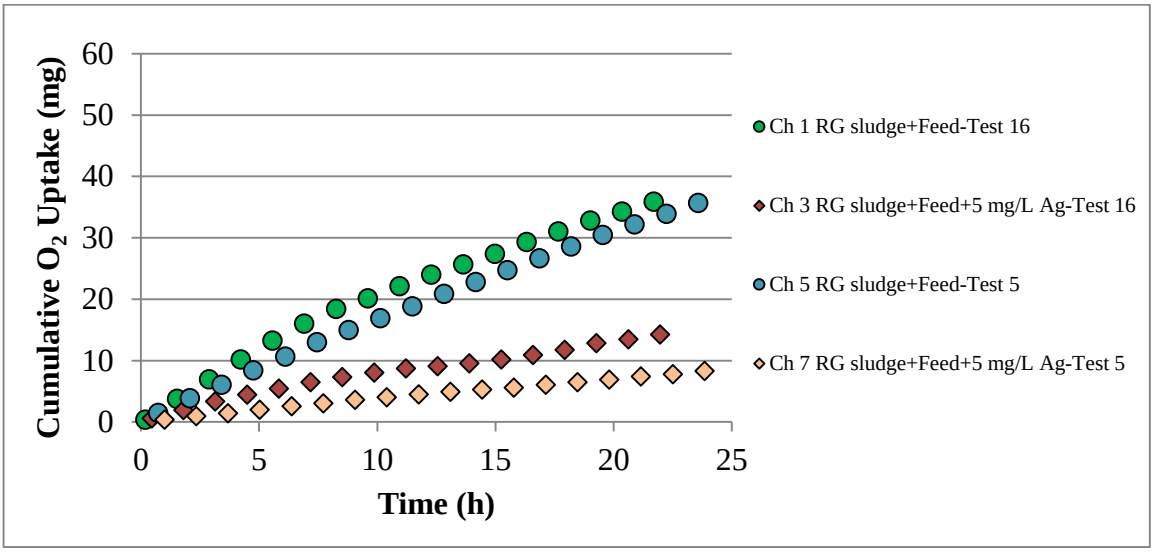

Figure 4.43. Results in Respirometric Test 5 and 16 (RG).

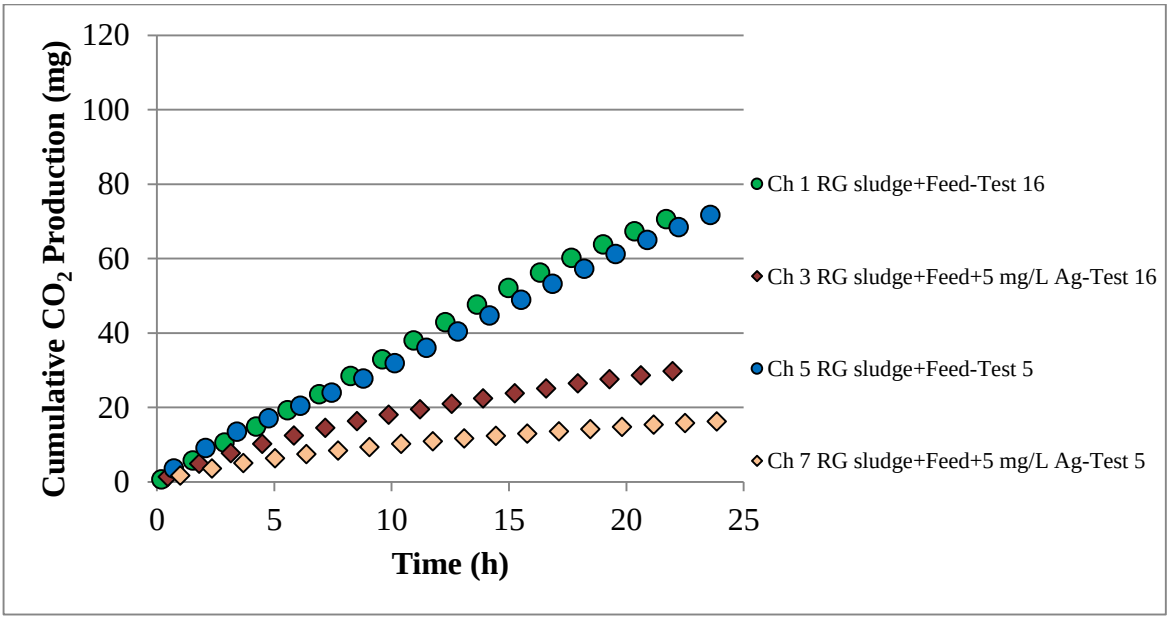

Figure 4.44. Results in Respirometric Test 5 and 16 (RG).

Overall, respirometry tests showed that while 2 mg/L Ag had a slight effect on RG sludge, higher Ag concentrations highly affected the performance of sludge. The percent inhibition due to Ag addition is presented in Table 4.7. Results show the average of respirometric tests.

Table 4.7. Inhibitory effect of Ag on RG sludge.

Ag Concentration (mg/L)	% Inhibition in T-O_2	% Inhibition in T-CO_2
2	5	12
3	78	38
4	68	43
5	85	69

4.2.2.3. Results of Peptone Reactor (RP): **Respirometric Tests 9, 13 and 17** were carried out at 2 mg/L Ag. Results of the **Test 13 and 17** were close to each other. Figure 4.45. shows that 2 mg/L Ag had no effect on RP sludge, as seen from total cumulative oxygen uptakes of control and metal-containing chambers that were close to each other.

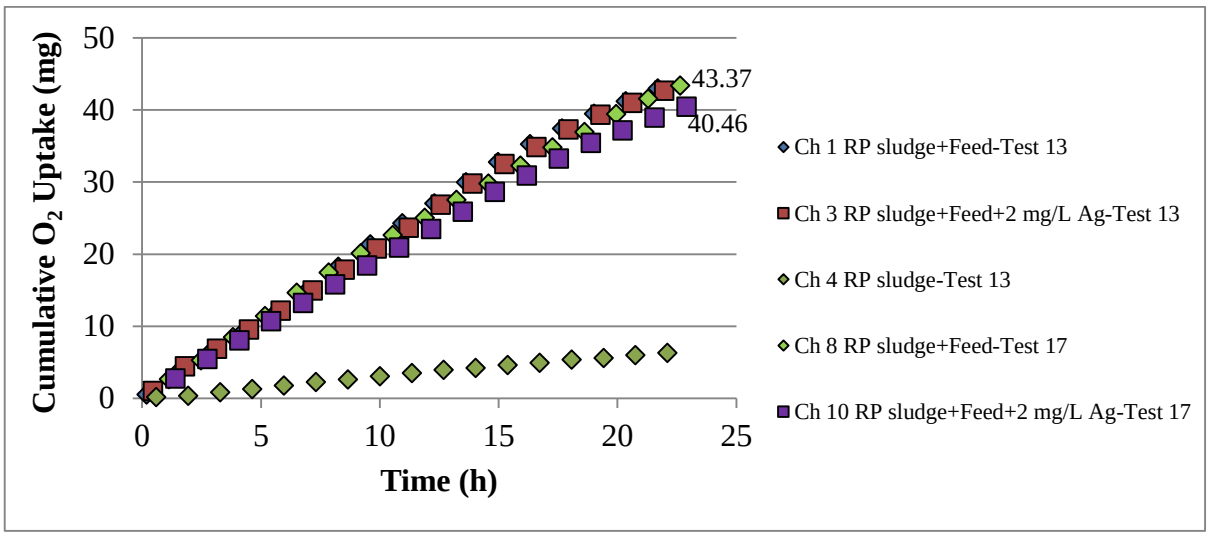

Figure 4.45. Results in Respirometric Test 13 and 17 (RP).

In **Test 9,** 2 mg/L Ag had no effect on RP sludge, but in this test cumulative O_2 uptakes were slightly higher compared to other two tests. The results are shown in Figure 4.46. The reason may be the MLVSS concentration. In **Test 9,** the initial MLVSS concentration was higher than other tests which may result in higher O_2 uptake.

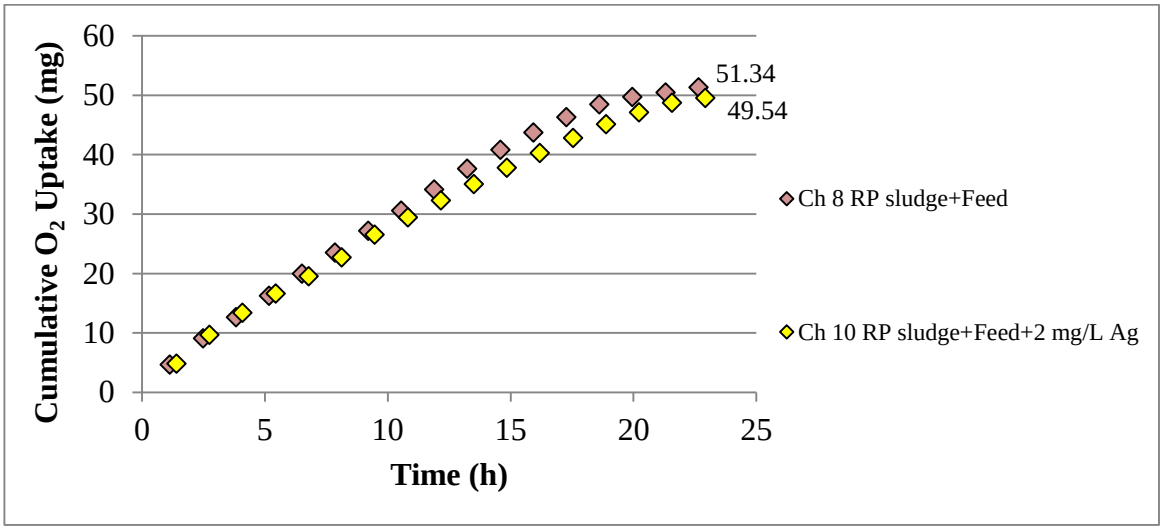

Figure 4.46. Results in Respirometric Test 9 (RP-05.08.2014).

Respirometric Test 15 were carried out at 3 mg/L Ag concentration. Results of this test are shown in Figure 4.47. As shown, 3 mg/L Ag had no effect on RP sludge, because total cumulative oxygen uptakes of control and metal-containing chambers were close to each other. Besides, Figure 4.48. shows cumulative carbon dioxide productions. This figure also indicates that 3 mg/L Ag did not affect the performance of the sludge as seen from cumulative CO_2 production of control and metal-containing chambers that were close to each other.

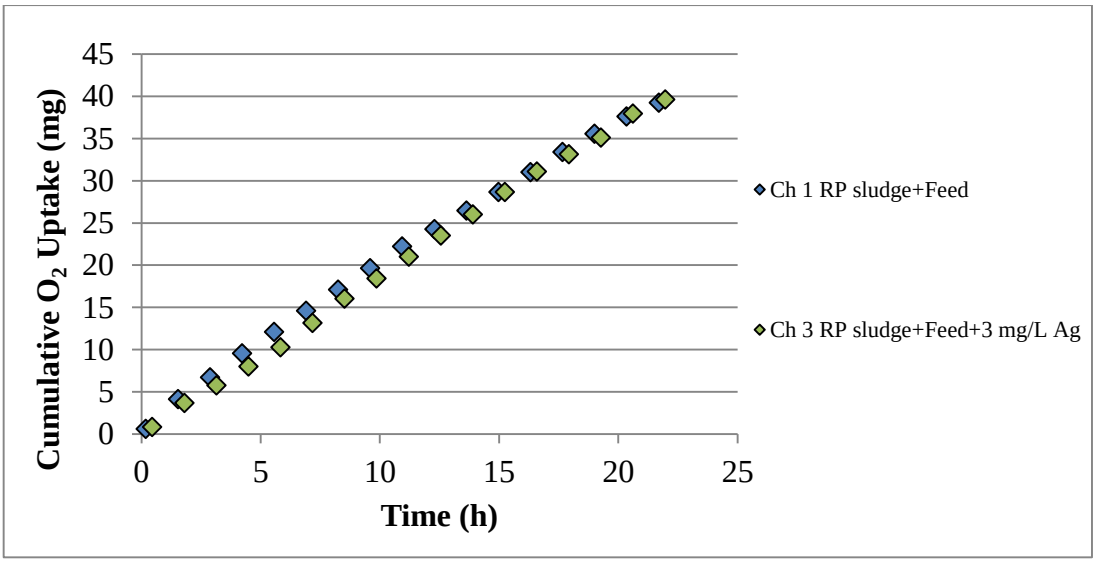

Figure 4.47. Results in Respirometric Test 15 (RP-28.08.2014).

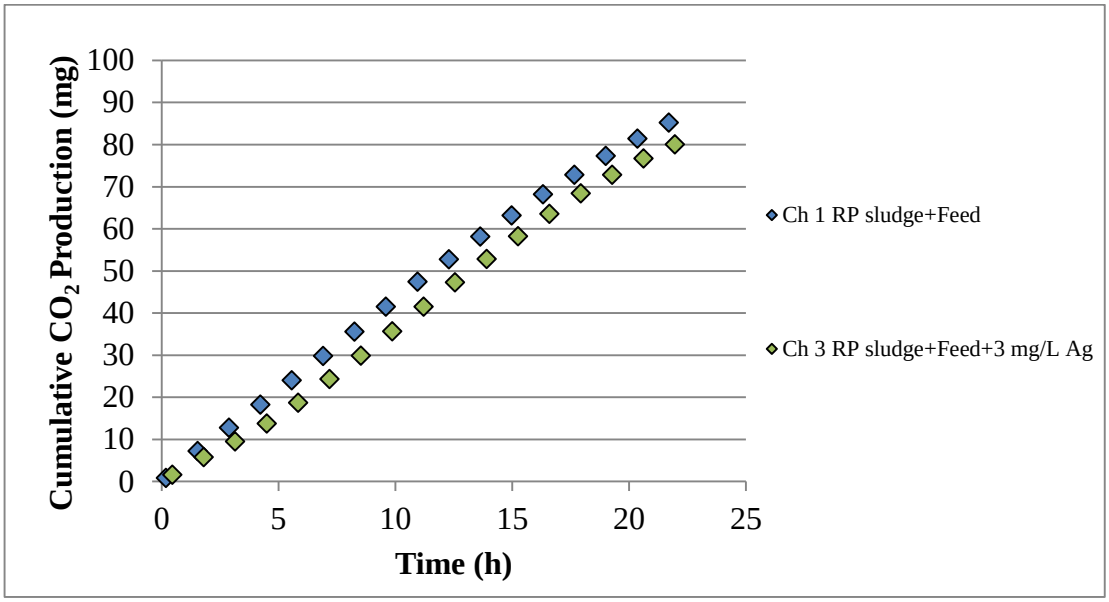

Figure 4.48. Results in Respirometric Test 15 (RP-28.08.2014).

Respirometric Tests 6, 11 and 14 were carried out at 4 mg/L Ag concentration. Results of these tests were close to each other. Figure 4.49. shows that 4 mg/L Ag had a slight inhibitory effect on this sludge. A small difference was observed between the total O_2 uptakes of control and metal-containing chambers. Besides, when oxygen uptake rates were analyzed, it was seen that uptake rates of control and metal-containing chambers were close to each other as seen in Figure 4.50.

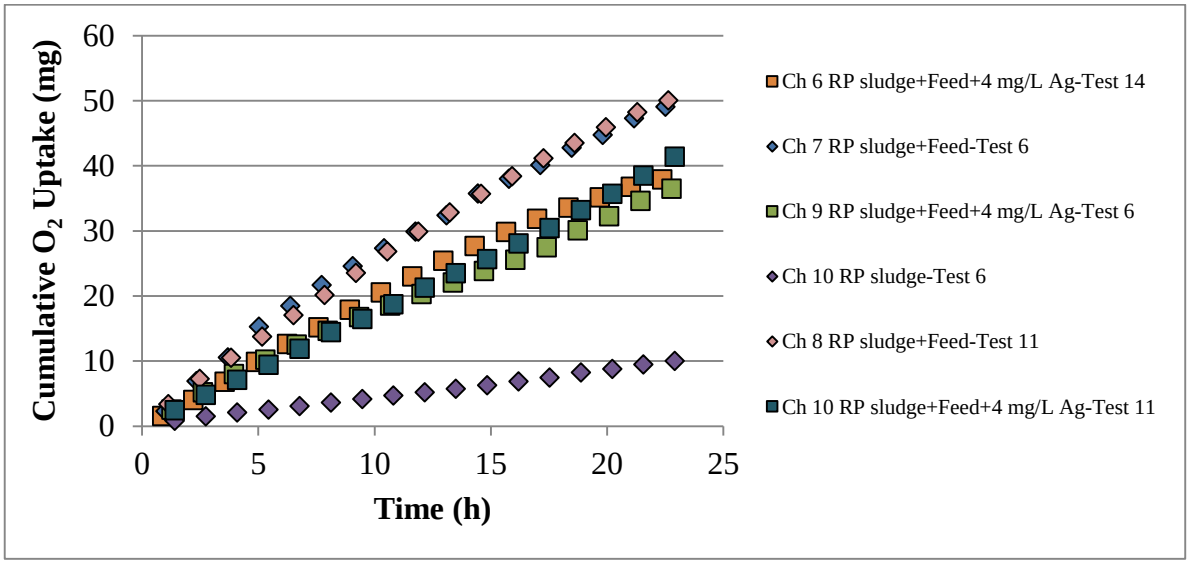

Figure 4.49. Results in Respirometric Test 6, 11 and 14 (RP).

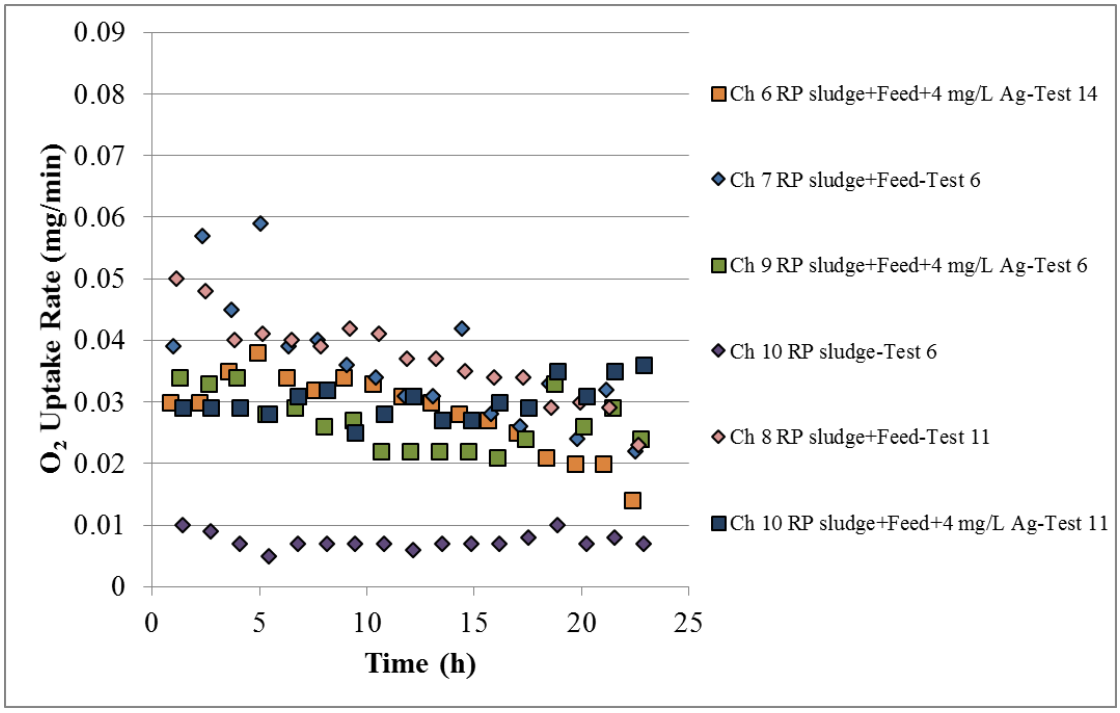

Figure 4.50. Oxygen uptake rates in Respirometric Test 6, 11 and 14 (RP).

Respirometric Tests 5, 12 and 16 were carried out at 5 mg/L Ag concentration. The results of **Respirometric Test 5 and 16** were close to each other. Figure 4.51. shows that 5 mg/L Ag had no effect on this sludge.

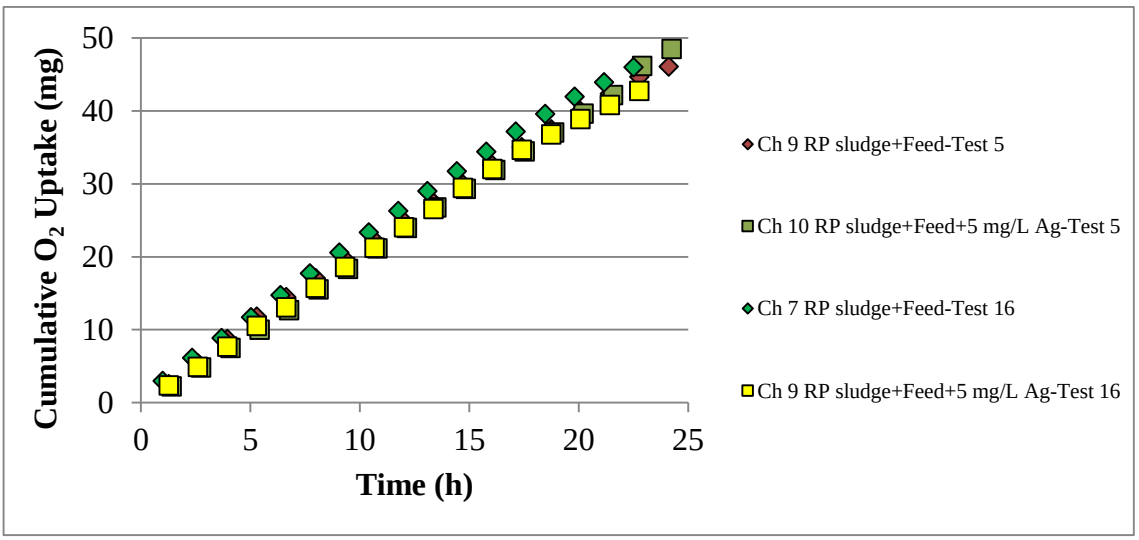

Figure 4.51. Results in Respirometric Test 5 and 16 (RP).

Additionally, in **Test 12** 5 mg/L had a slight inhibitory effect on RP sludge. Results are shown in Figure 4.52. The reason may be the lower MLVSS concentration compared to other tests. Also, the total O_2 uptake in the control chamber was lower compared to the other tests.

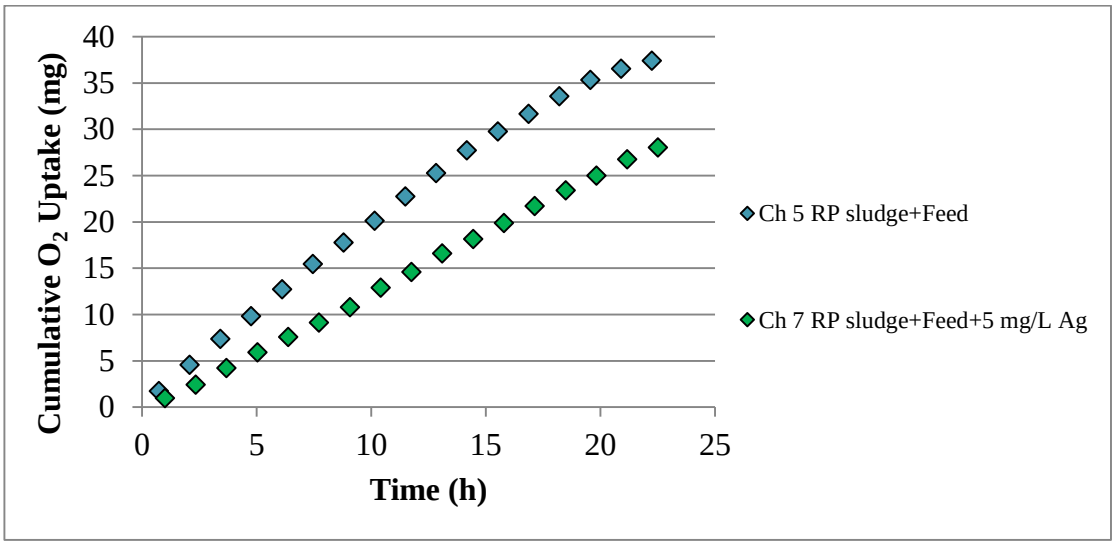

Figure 4.52. Results in Respirometric Test 12 (RP-19.08.2014).

Overall, respirometry tests showed that in the range of 2–5 mg/L Ag had no inhibitory effect on RP sludge. In the presence of Ag, oxygen uptake or carbon dioxide generation in the RP sludge was not affected. The percent inhibition due to Ag addition is presented in Table 4.8. Results show the average of respirometric tests.

Table 4.8. Inhibitory effect of Ag on RP sludge.

Ag Concentration (mg/L)	% Inhibition in T-O_2	% Inhibition in T-CO_2
2	4	7
3	2	3
4	15	22
5	8	8

According to these results, it was thought that RP sludge was not affected by addition of Ag because of feed composition. It is believed that proteins in peptone form complexes with Ag. In order to determine the effect of Feed P, two additional respirometry tests were carried out. In the first test, RP sludge was used. Either Feed P or Feed G were added in the presence of 3 mg/L and 5 mg/L Ag. The results are shown in Figure 4.53. and Figure 4.54.

RP sludge which was fed with Feed P was not affected by Ag as seen from Figure 4.53. However, the same sludge when fed with Feed G was inhibited at 3 mg/L Ag. In addition, 5 mg/L Ag totally inhibited the sludge fed with Feed G as seen from total O_2 uptake which was 0 mg as shown in Figure 4.54.

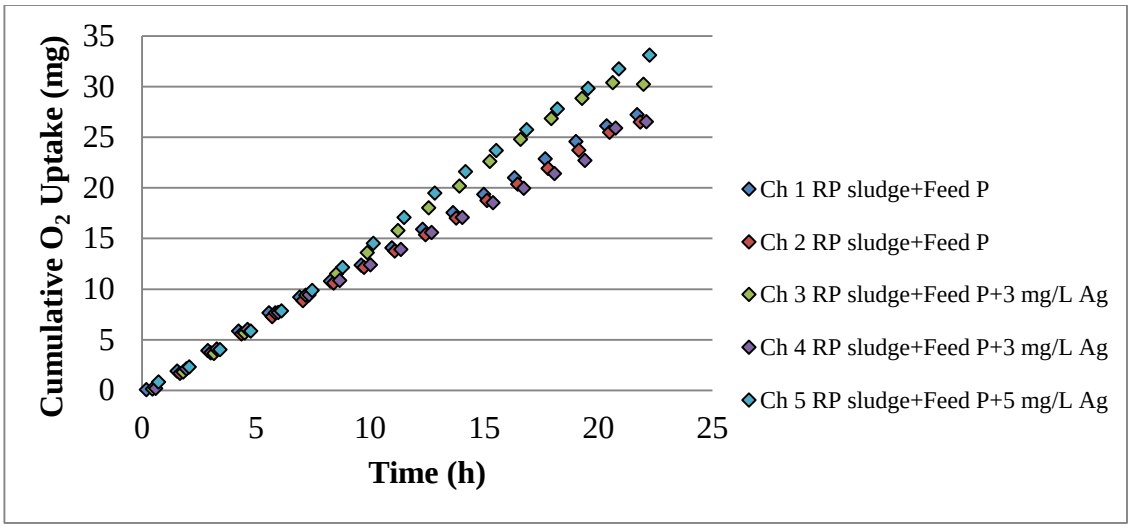

Figure 4.53. Results in Respirometric Test 18 (RP-20.10.2014).

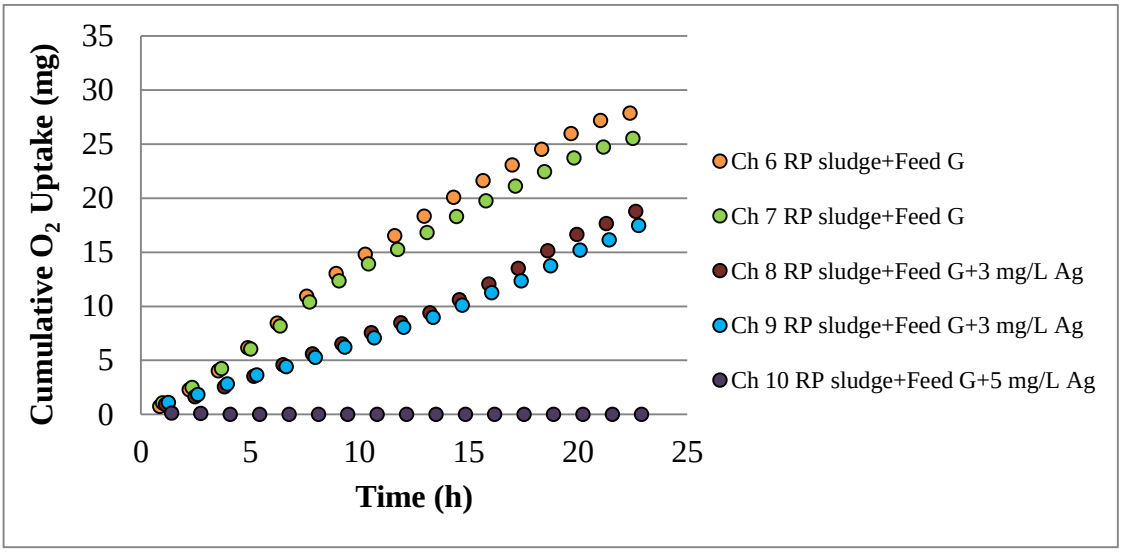

Figure 4.54. Results in Respirometric Test 18 (RP-20.10.2014).

The second test was carried out with the sludge taken from the RG reactor. Also in this test, Feed G and Feed P were used. Figure 4.55. shows that the sludge fed with Feed G was highly inhibited at 3 mg/L and 5 mg/L Ag. However, Figure 4.56. shows that the performance of the sludge fed with Feed P was not affected by Ag addition.

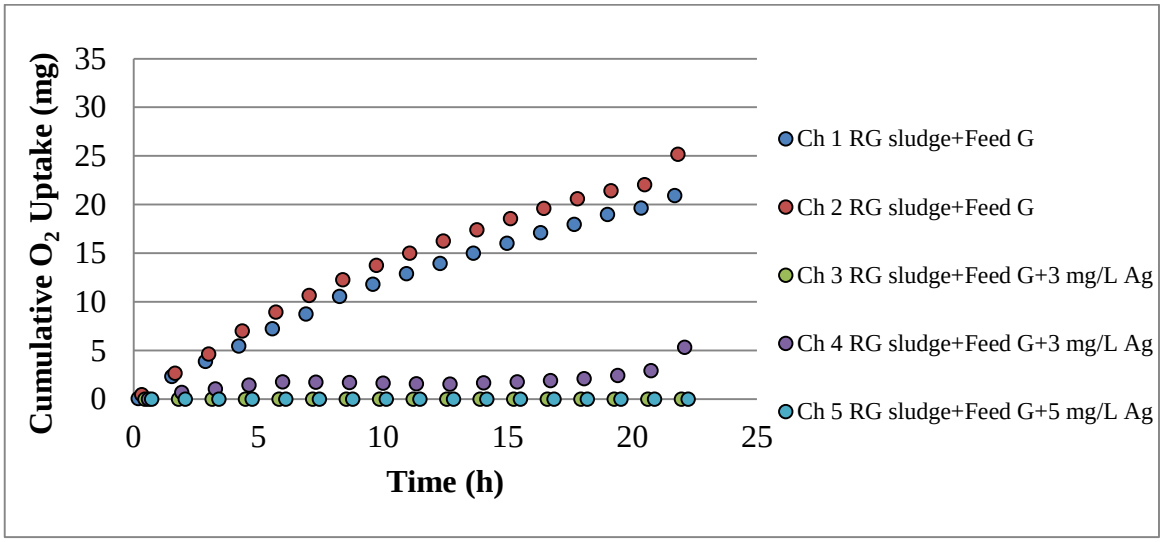

Figure 4.55. Results in Respirometric Test 19 (RG-27.10.2014).

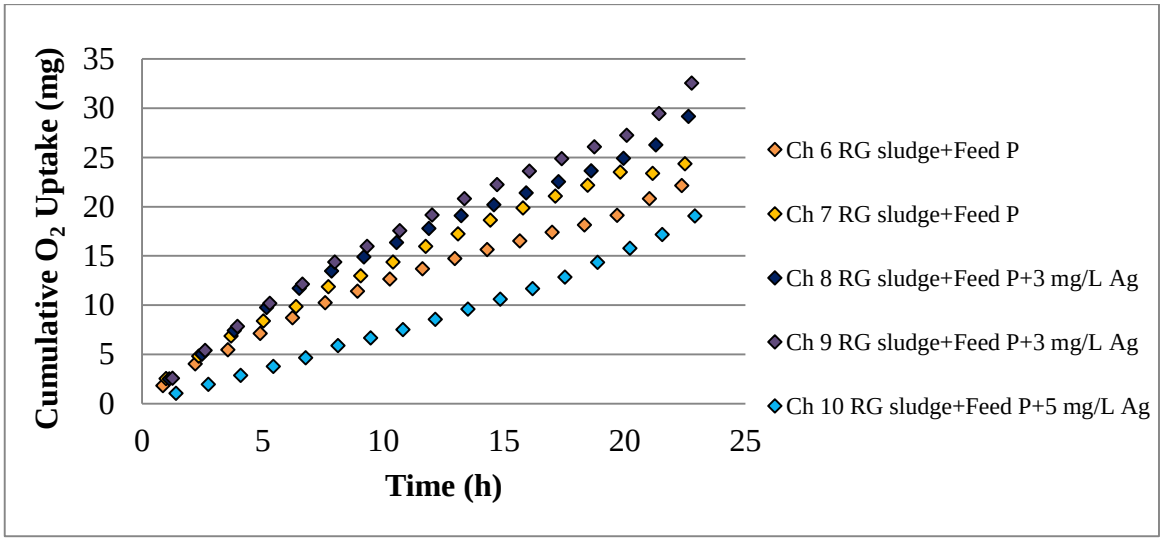

Figure 4.56. Results in Respirometric Test 19 (RG-27.10.2014).

The results of these two tests showed that when the sludge was fed with Feed P, it was not affected by the addition of Ag. This indicates that in the presence of Feed P, inhibitory effect of Ag was reduced. Obviously, peptone contains organic substances like proteins which may form complexes with the Ag ion. As a result, sludge performance was not affected.

4.2.2.4. Inhibitory Effect of Ag on Reactor 3 (R3): As a complementary work for the former study (Ayyıldız, 2013), a respirometry test was carried out with R3 sludge. 0.25, 0.75 and 1

mg/L Ag and ATU was used for this test. Results of **Test 20** are shown in Figure 4.57. According to this figure, oxygen uptake was zero mg in chambers which contained 0.75 and 1 mg/L Ag. This indicates that R3 sludge was totally affected. Moreover, Figure 4.58. shows the cumulative carbon dioxide production. According to this figure, while 0.25 mg/L Ag slightly affected the performance of R3 sludge, 0.75 and 1 mg/L Ag affected this sludge about 50% in terms of carbon dioxide production. Since in R3 sludge nitrifiers had a high fraction, this sludge was easily affected by Ag even at low concentrations. The results of this test are in accordance with the former study (Ayyıldız, 2013).

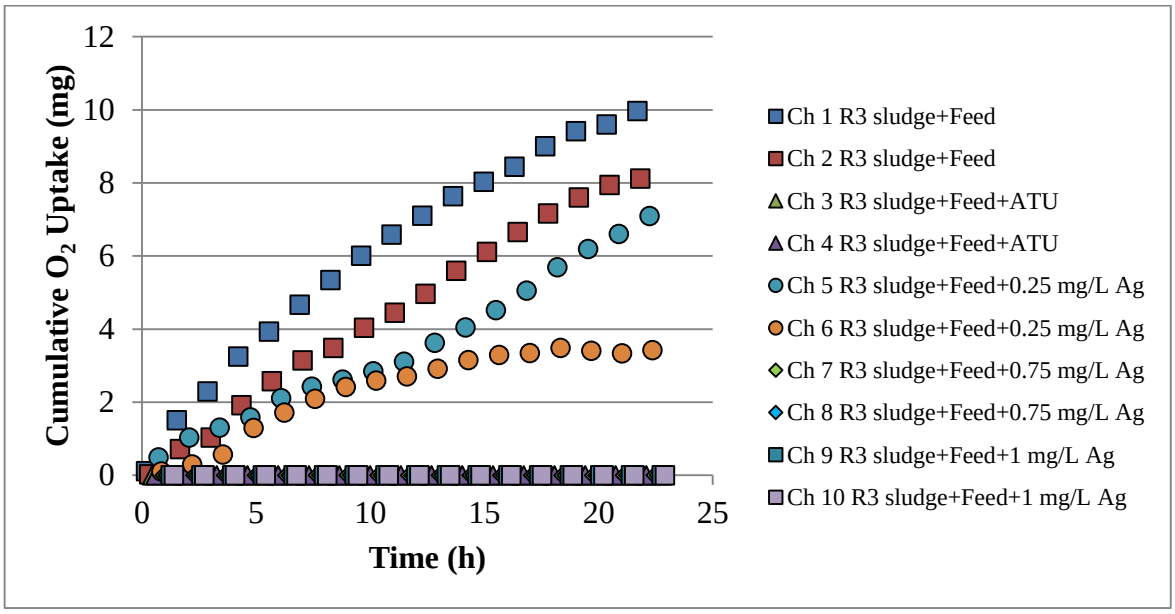

Figure 4.57. Results in Respirometric Test 20 (R3-10.11.2014).

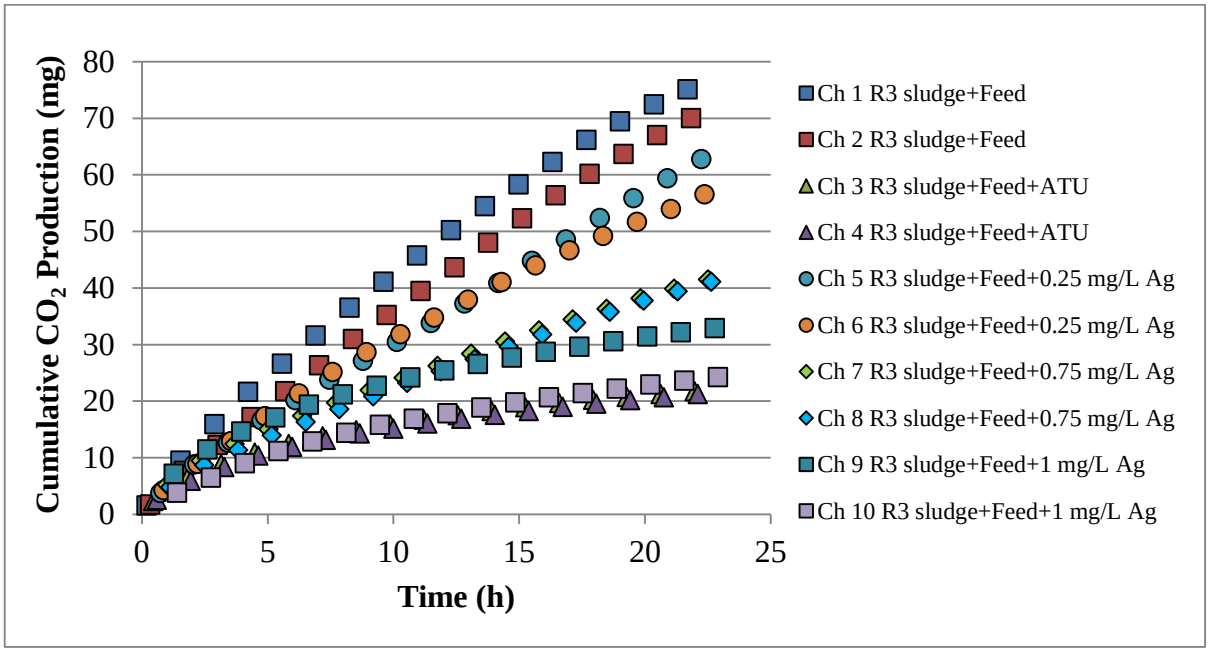

Figure 4.58. Cumulative CO_2 Production in Respirometric Test 20 (R3-10.11.2014).

The percent inhibition due to Ag addition is presented in Table 4.9. According to the table, 0.75 and 1 mg/L Ag totally affected the oxygen uptake of R3 sludge, while carbon dioxide production was highly affected. The percentages of inhibitions in T-O_2 and T-CO_2 at different concentrations were presented in Figure 4.59. and Figure 4.60., respectively.

Table 4.9. Inhibitory effect of Ag on R3 sludge.

Ag Concentration (mg/L)	% Inhibition in T-O_2	% Inhibition in T-CO_2
0.25	42	18
0.75	100	43
1	100	61

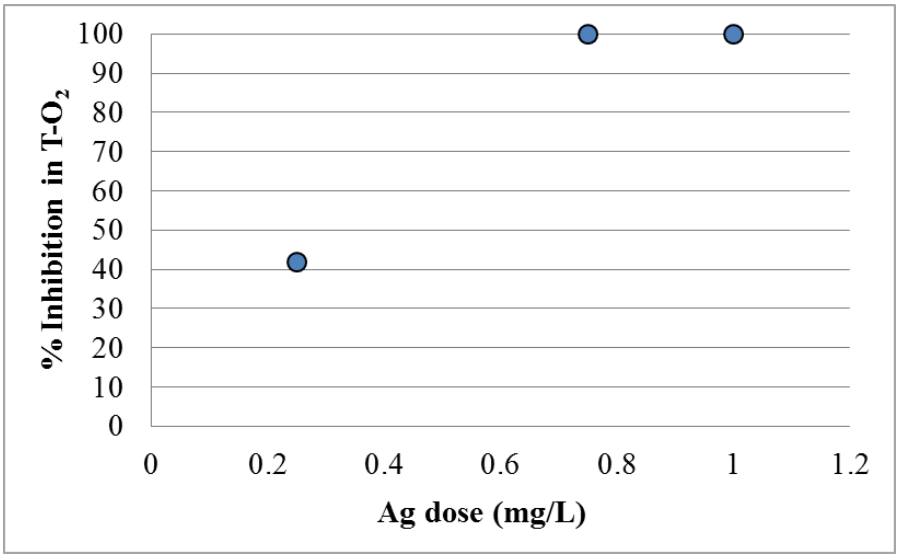

Figure 4.59. The percentage of inhibition in T-O_2 at different Ag concentrations on R3.

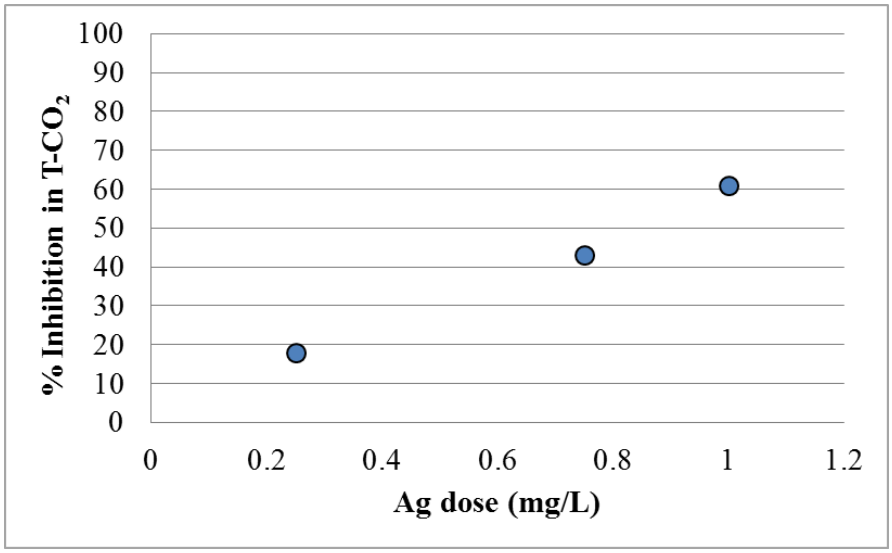

Figure 4.60. The percentage of inhibition in T-CO_2 at different Ag concentrations on R3.

4.2.3. Overall Evaluation of the Inhibitory Effect of Ag on CR, RG and RP

In order to show the total effect of all concentrations, the percentage of inhibition in T-O_2 was presented in following figures. These figures included the standard deviation of respirometric tests. Total oxygen uptake is the sum of carbonacous and nitrogenous oxygen uptake. The contribution of N-O_2 to T-O_2 in CR, RG and RP sludges was calculated on the average as 27%, 21% and 37%, respectively. According to these figures, CR and RG sludges were highly affected after 3 mg/L Ag concentration, while RP sludge was slightly affected by the addition of Ag.

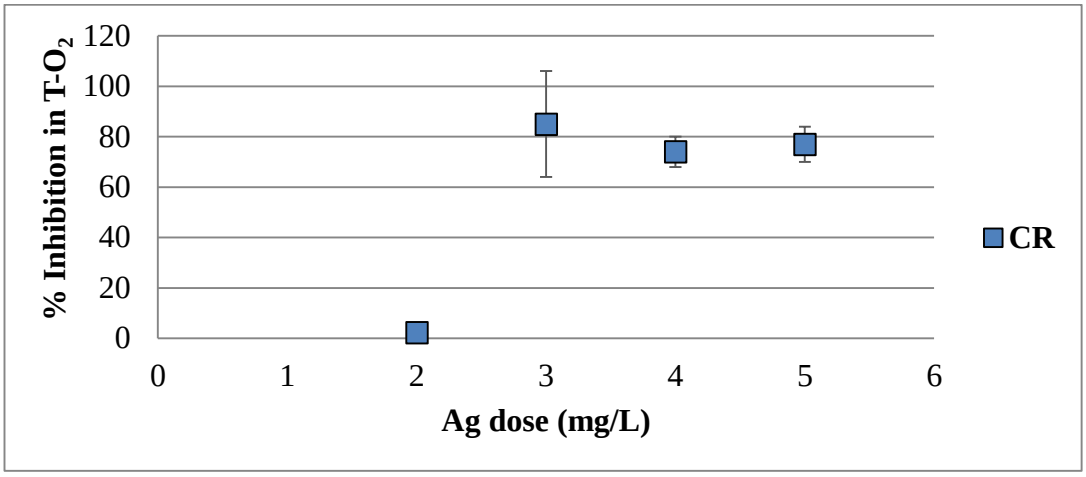

Figure 4.61. The percentage of inhibition in T-O_2 at different Ag concentrations in CR.

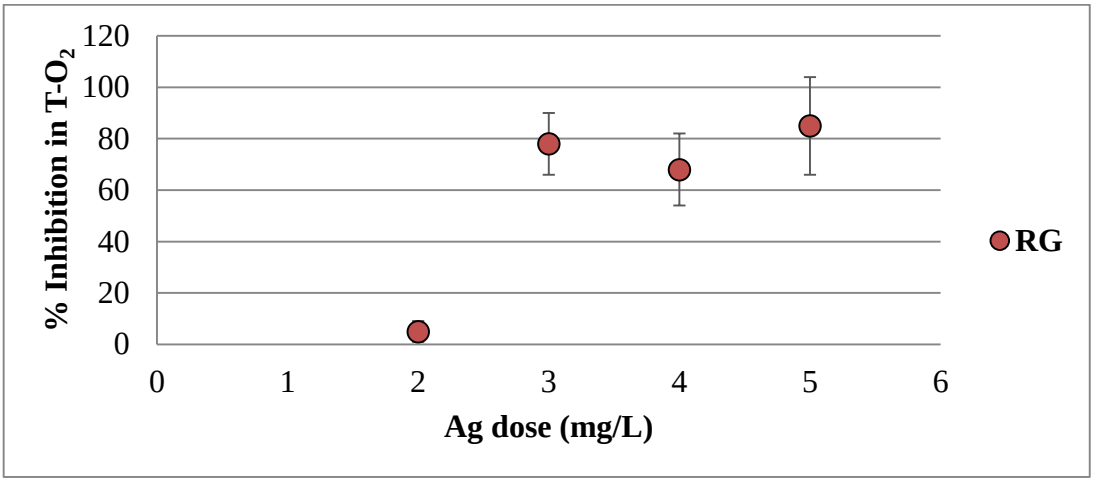

Figure 4.62. The percentage of inhibition in T-O_2 at different Ag concentrations in RG.

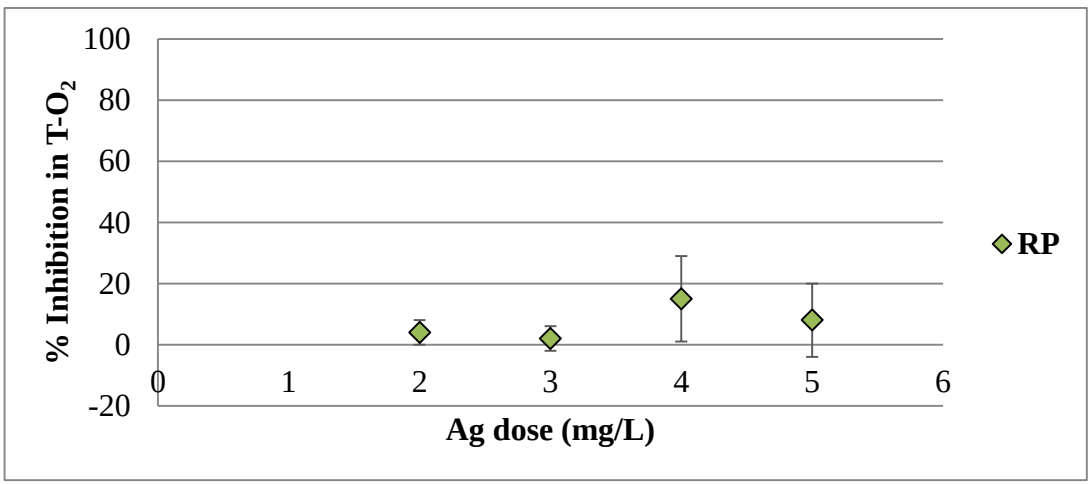

Figure 4.63. The percentage of inhibition in T-O_2 at different Ag concentrations in RP.

Moreover, the percentage of inhibition in T-CO_2 is also presented in Figure 4.64., Figure 4.65. and Figure 4.66. Total carbon dioxide production in CR sludge was highly affected after 3 mg/L Ag, while 5 mg/L Ag highly affected RG sludge. However, in RP sludge total carbon dioxide production was slightly affected by the addition of Ag.

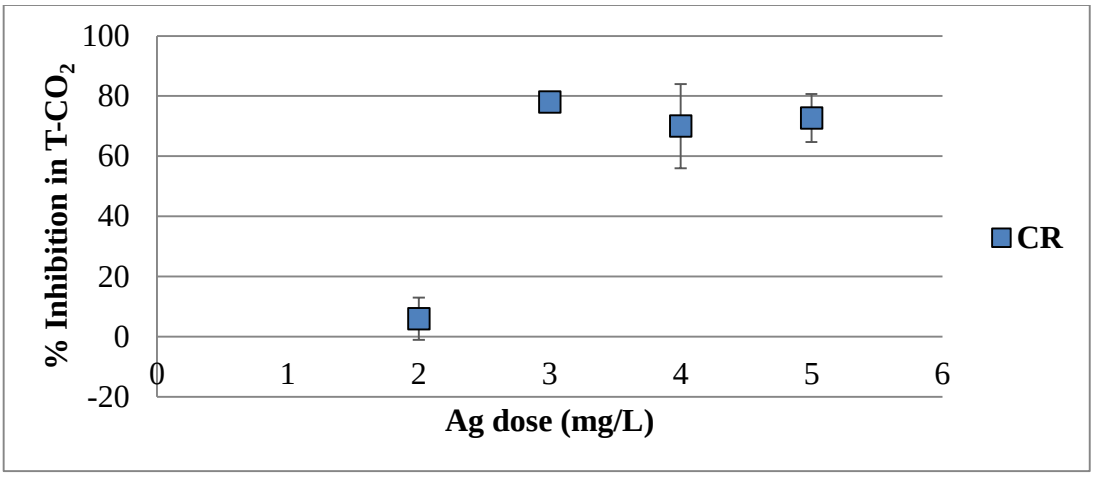

Figure 4.64. The percentage of inhibition in T-CO_2 at different Ag concentrations in CR.

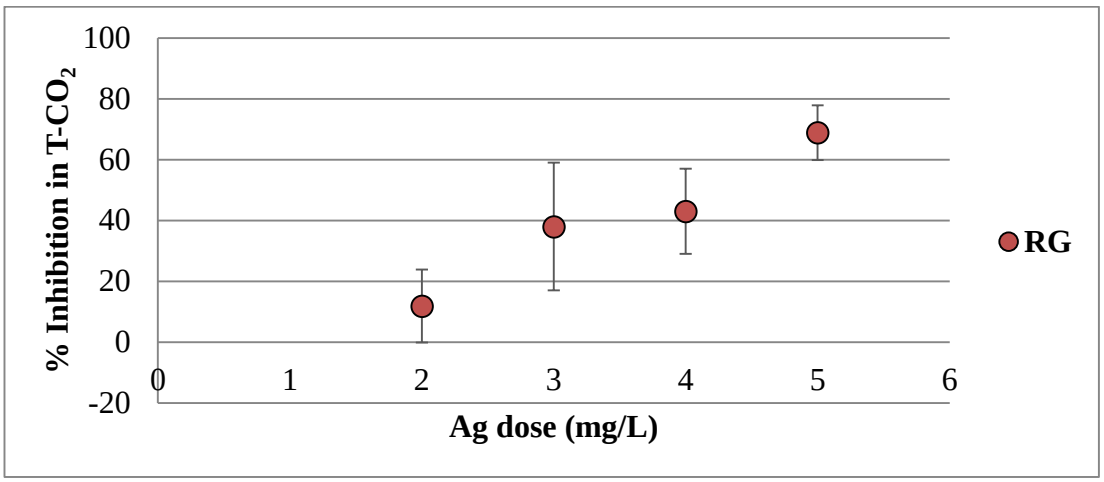

Figure 4.65. The percentage of inhibition in T-CO_2 at different Ag concentrations in RG.

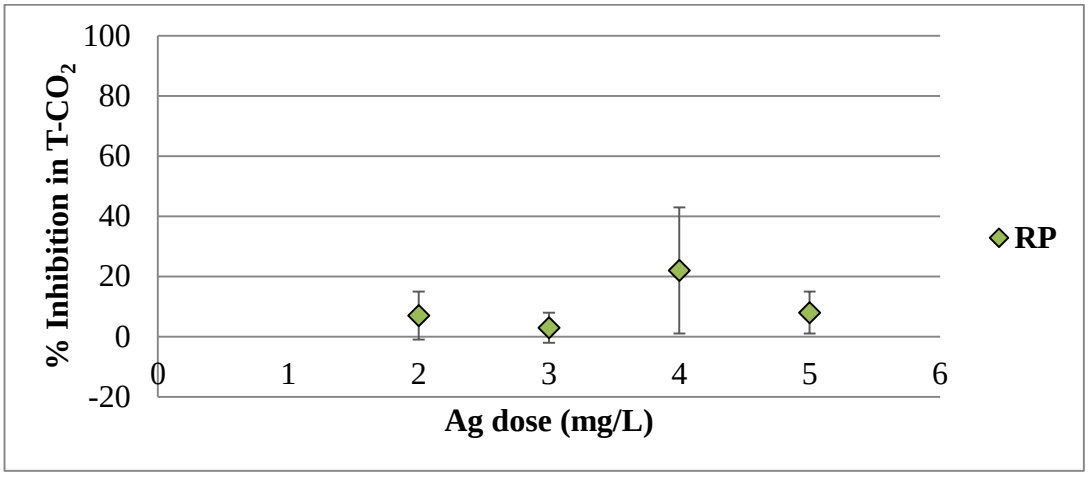

Figure 4.66. The percentage of inhibition in T-CO_2 at different Ag concentrations in RP.

The percentage of inhibition in C-O_2 and N-O_2 in these sludges was also presented in following figures. These figures show that carbonaceous oxygen uptake in CR and RG sludges was affected by 3, 4 and 5 mg/L Ag concentration, but carbonaceous oxygen uptake in RP sludge was not affected at these concentrations. On the other hand, nitrogenous oxygen uptake in these sludges was affected more (approximately 60%) than carbonaceous oxygen uptake.

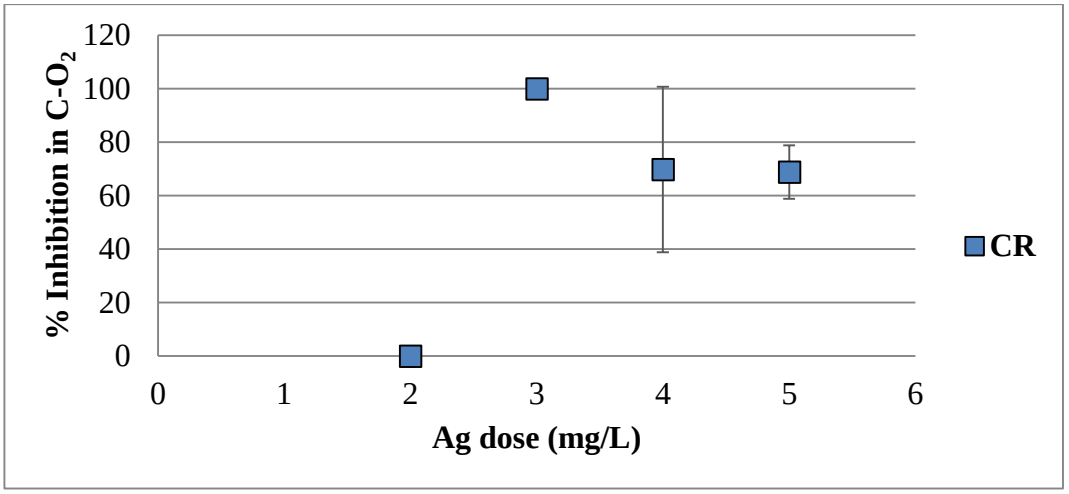

Figure 4.67. The percentage of inhibition in C-O_2 at different Ag concentrations in CR.

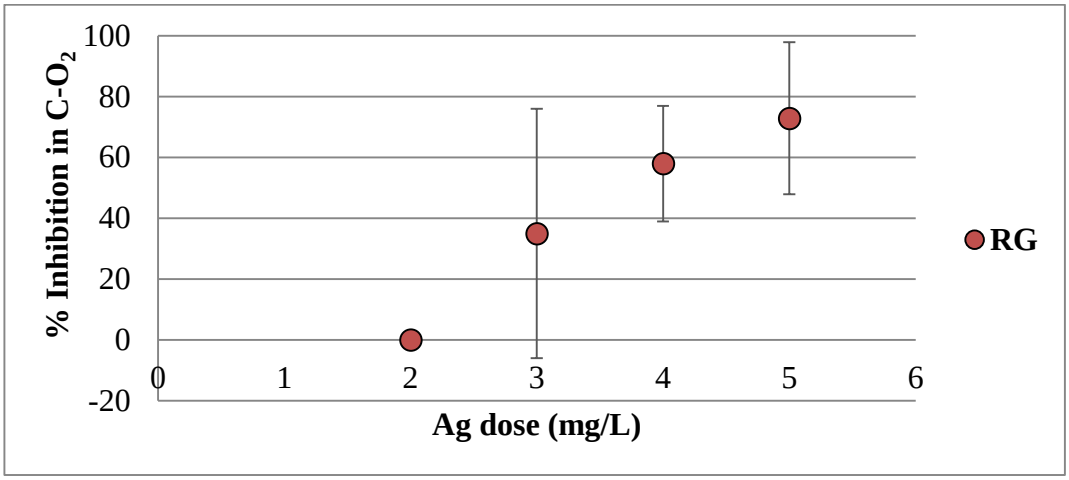

Figure 4.68. The percentage of inhibition in C-O_2 at different Ag concentrations in RG.

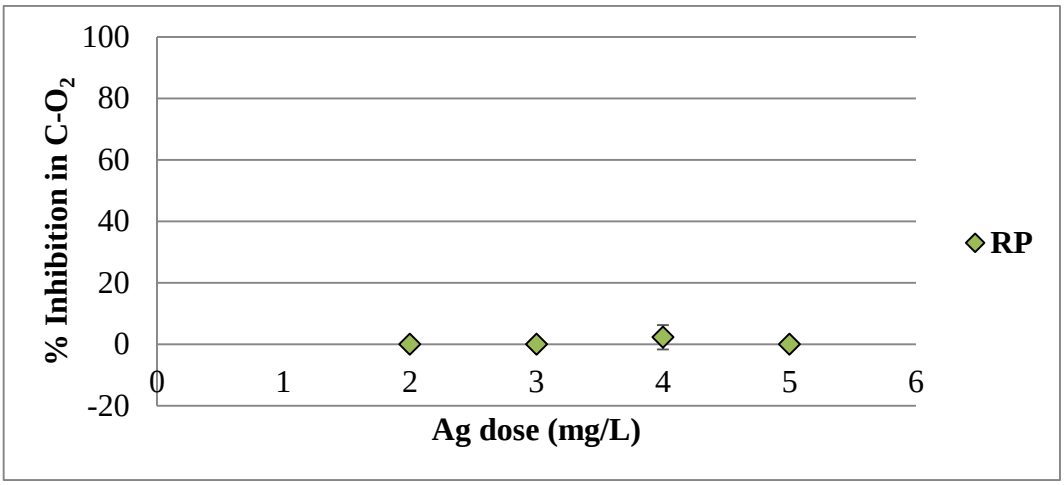

Figure 4.69. The percentage of inhibition in C-O_2 at different Ag concentrations in RP.

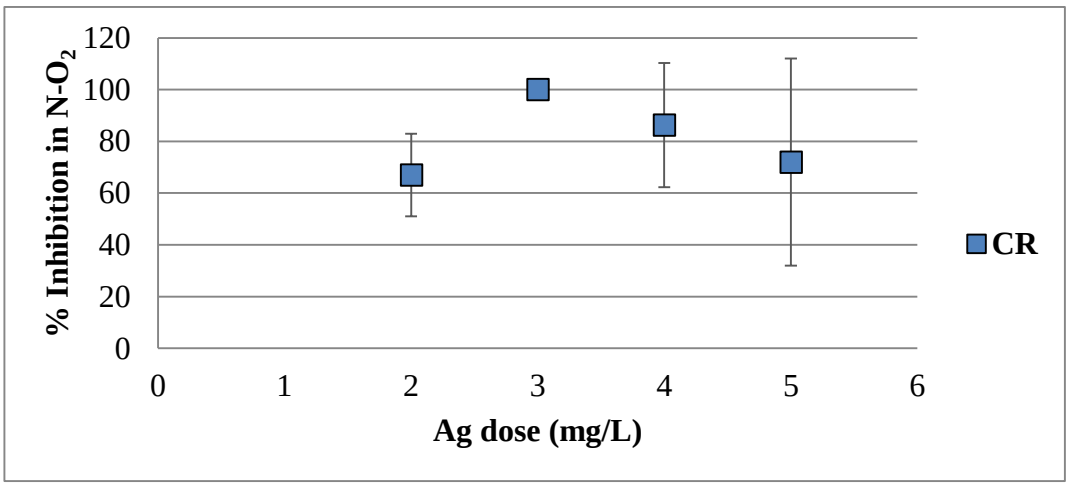

Figure 4.70. The percentage of inhibition in N-O_2 at different Ag concentrations in CR.

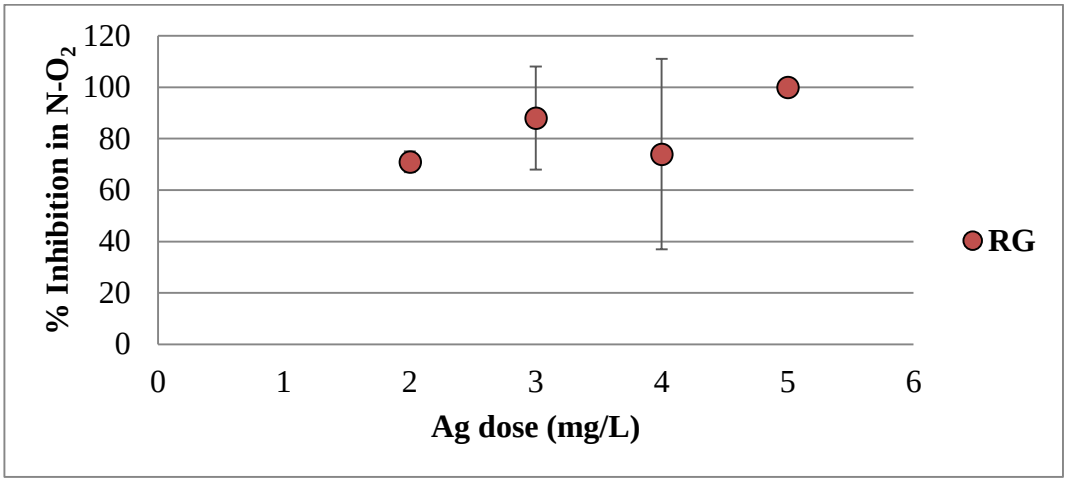

Figure 4.71. The percentage of inhibition in $N\text{-}O_2$ at different Ag concentrations in RG.

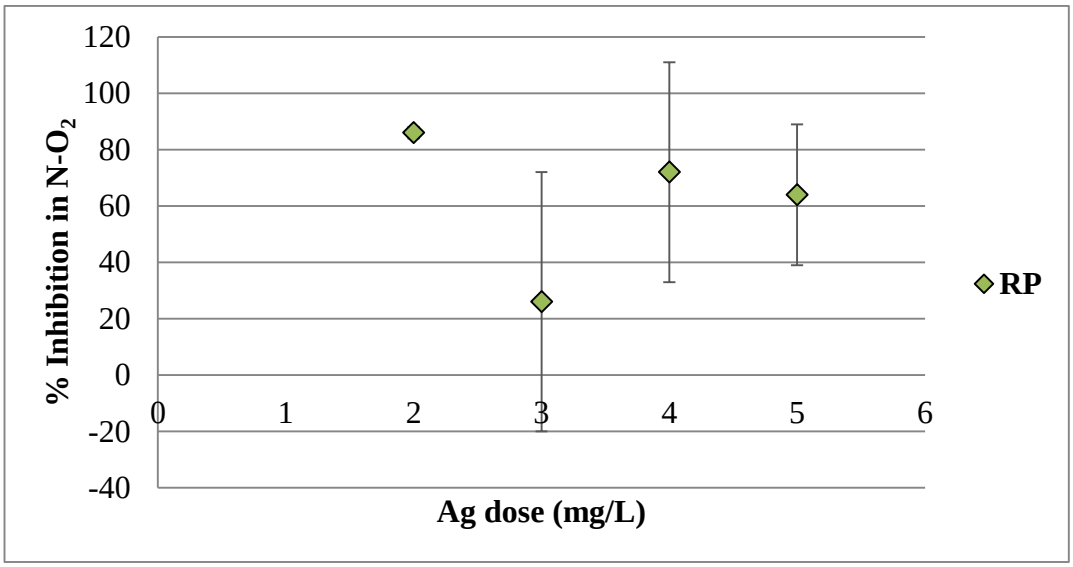

Figure 4.72. The percentage of inhibition in $N\text{-}O_2$ at different Ag concentrations in RP.

Additionally, the percentage of inhibition in $C\text{-}CO_2$ and $N\text{-}CO_2$ in these sludges was presented in Figure 4.73., Figure 4.74., Figure 4.75., Figure 4.76., Figure 4.77. and Figure 4.78. Figure 4.73., Figure 4.74. and Figure 4.75. show that CR and RG sludges were highly affected after 3 mg/L Ag concentration in terms of $C\text{-}CO_2$. Besides, Ag had almost no effect on carbonaceous CO_2 production in RP sludge. The percentage of inhibition in $N\text{-}CO_2$ shows that CR sludge was not affected at 2 mg/L Ag concentration, while it was highly affected at 3, 4 and 5 mg/L Ag. In addition, RG and RP sludges were highly affected at all Ag concentrations.

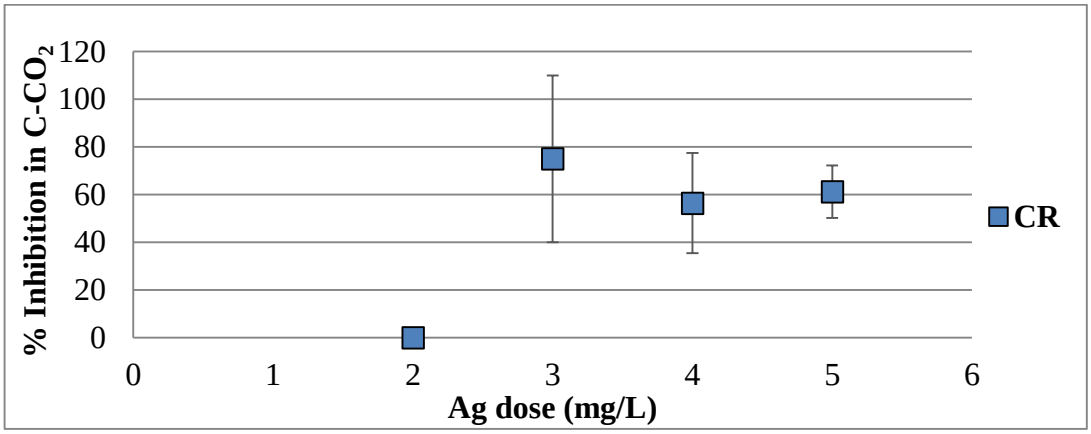

Figure 4.73. The percentage of inhibition in C-CO_2 at different Ag concentrations in CR.

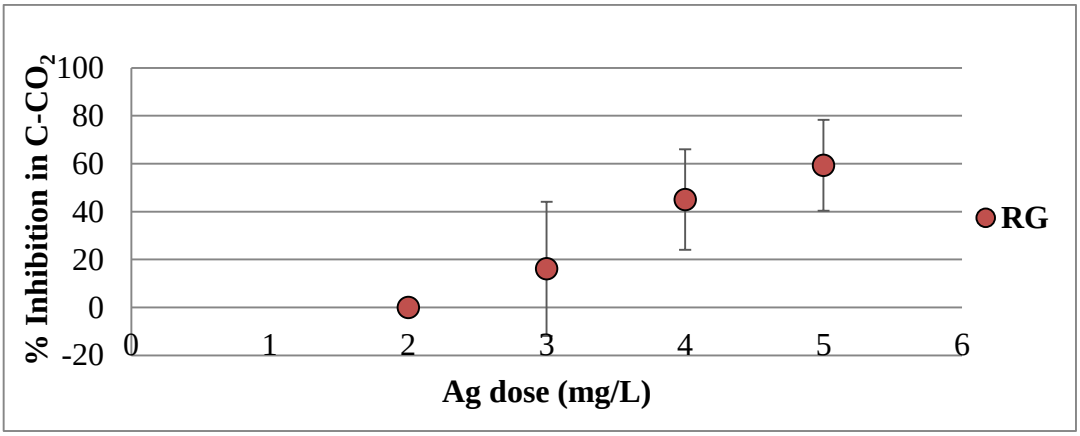

Figure 4.74. The percentage of inhibition in C-CO_2 at different Ag concentrations in RG.

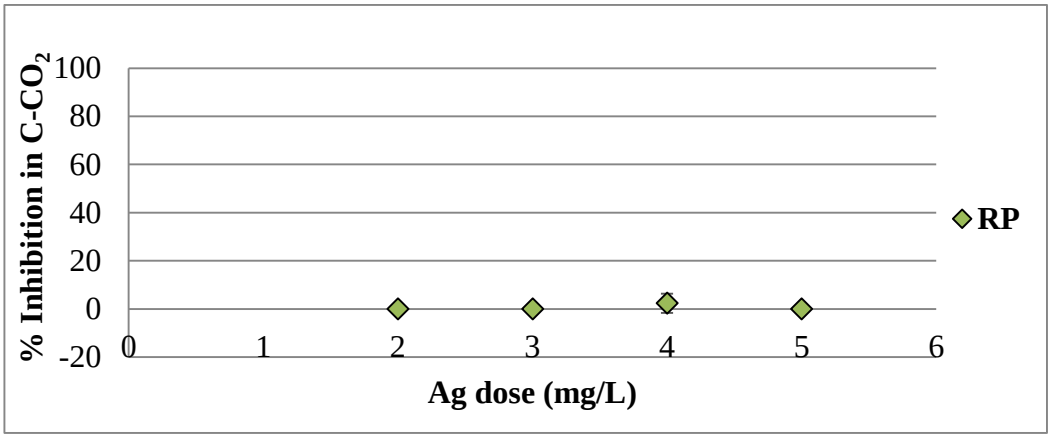

Figure 4.75. The percentage of inhibition in C-CO_2 at different Ag concentrations in RP.

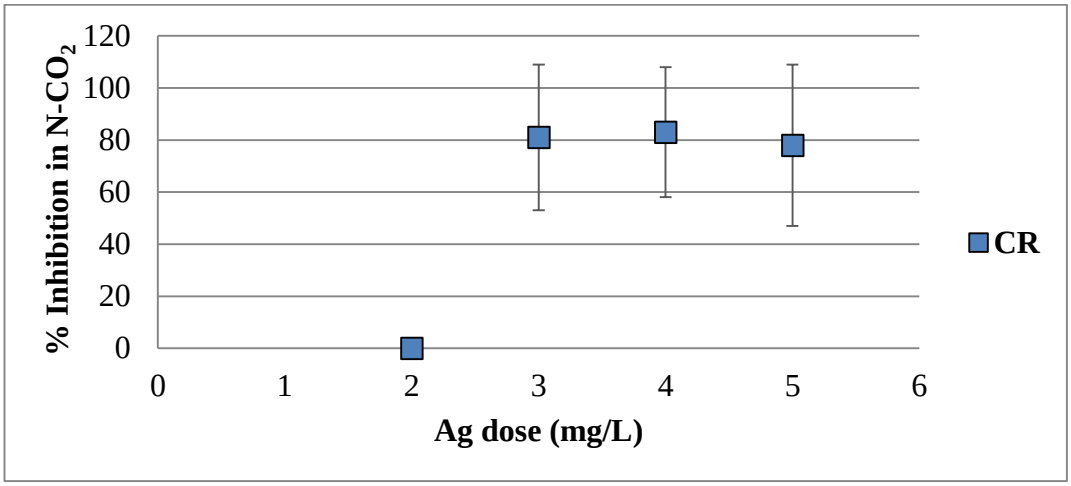

Figure 4.76. The percentage of inhibition in $N\text{-}CO_2$ at different Ag concentrations in CR.

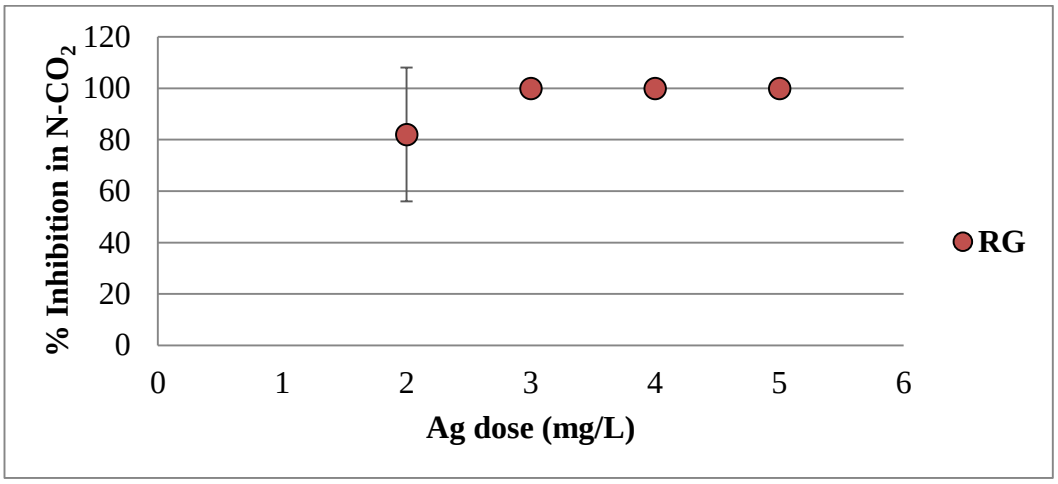

Figure 4.77. The percentage of inhibition in $N\text{-}CO_2$ at different Ag concentrations in RG.

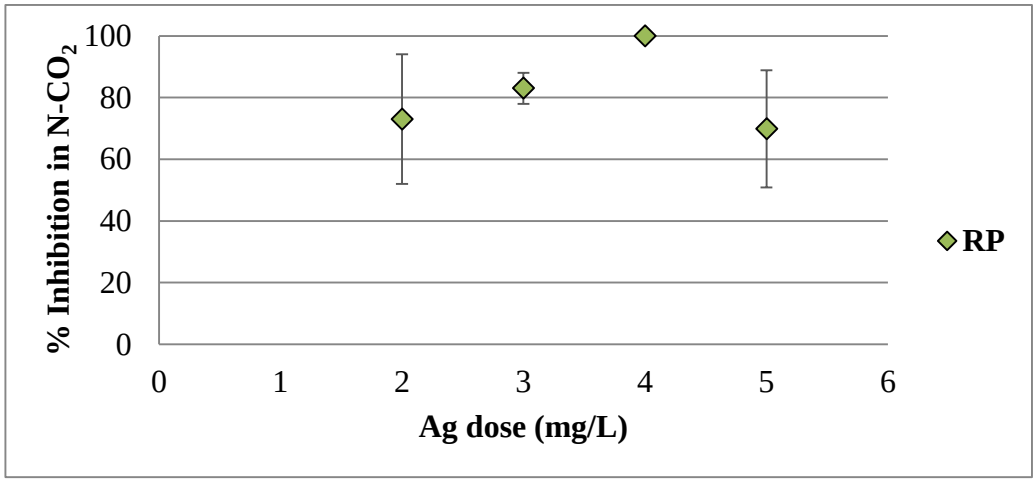

Figure 4.78. The percentage of inhibition in $N\text{-}CO_2$ at different Ag concentrations in RP.

4.3. Surface Charges and Hydrophobicities of Different Sludges

Initially, surface charge and hydrophobicity analyses were done before and after semi-continuous feeding to examine the effect of starvation conditions. Table 4.10. shows the results before and after feeding. The details are given in the Materials and Methods section. Results showed that surface charges and hydrophobicities of sludges were similar before and after feeding. Therefore, when respirometric tests were started, surface charge and hydrophobicity analyses were only carried out before each test.

Table 4.10. Surface charges and hydrophobicities before and after feeding of sludges.

Sludge	**Surface charge (meqv/g MLSS)**				**Hydrophobicity (%)**			
	Date	**Before Feeding**	**Date**	**After Feeding**	**Date**	**Before Feeding**	**Date**	**After Feeding**
CR	11.4.2014	-0.061	9.4.2014	-0.055	11.4.2014	50	9.4.2014	49
	30.4.2014	-0.062	28.4.2014	-0.058	30.4.2014	37	28.4.2014	41
			6.6.2014	-0.063			6.6.2014	53
RG	21.3.2014	-0.069	19.3.2014	-0.081	21.3.2014	67	19.3.2014	62
	9.5.2014	-0.084	7.5.2014	-0.081	9.5.2014	63	7.5.2014	58
			6.6.2014	-0.058			6.6.2014	29
RP	21.3.2014	-0.087	19.3.2014	-0.072	21.3.2014	49	19.3.2014	56
			2.4.2014	-0.046			2.4.2014	49
			10.4.2014	-0.047			10.4.2014	52
			6.6.2014	-0.050			6.6.2014	65

All surface charges and hydrophobicities are shown in Figure 4.79. and Figure 4.80. with respect to time. The starting date of experiments (t=261st day) was 19th March 2014. According to Figure 4.79., it can be seen that CR and RG sludge had similar surface charges. On the other hand, RP sludge had the lowest surface charge which varied between -0.08 and -0.04 meqv/g MLSS. Average surface charges of CR, RG and RP were found as -0.084±0.02, -0.094±0.02 and -0.060±0.01 meqv/g MLSS, respectively. Additionally, t-test was applied for the surface charges of CR, RG and RP. Results are given in Table 4.11. This table indicates that CR and RG sludges had close surface charges, because the p value of the t-test was higher than 0.05. On the other hand, RG and RP as well as CR and RP were different from each other as seen from very low p values.

Table 4.11. Results of t-test for surface charge.

Comparison of Reactors	p value
CR-RG	0.701898
CR-RP	0.000142
RG-RP	0.00000067

According to some studies, it is believed that the negative surface charge of sludge results from the carbohydrate content (Liao et al., 2001; Vatansever, 2005). Since RG was fed with glucose only as an organic substrate, it had a higher negative surface charge. Moreover, also CR sludge had a relatively high negative surface charge similar to RG. The differences may be explained with the EPS characteristics of the sludges.

Results of a Ph.D. study in progress, showed that Tightly bound EPS (TB-EPS) was dominant compared to Soluble EPS (SEPS) and Loosely bound EPS (LB-EPS) in CR, RG and RP sludges (Geyik, 2014). In this ongoing study, it was observed that in all sludges protein-EPS was more dominant. However, the highest protein to carbohydrate ratio was observed in the EPS of RP sludge, while the lowest value was seen in the EPS of RG. RG sludge was fed with only glucose (a carbohydrate), so the carbohydrate amount in EPS was found higher as expected. In addition, the total EPS production was found to be similar to CR sludge. Carbohydrate-EPS was at the lowest value in RP sludge, because it was only fed with peptone which is a mixture consisting largely of proteins. As a result, it was concluded that EPS fractions change in the presence different substrates (TÜBİTAK Project Report No: 5, 2014; Geyik, 2014).

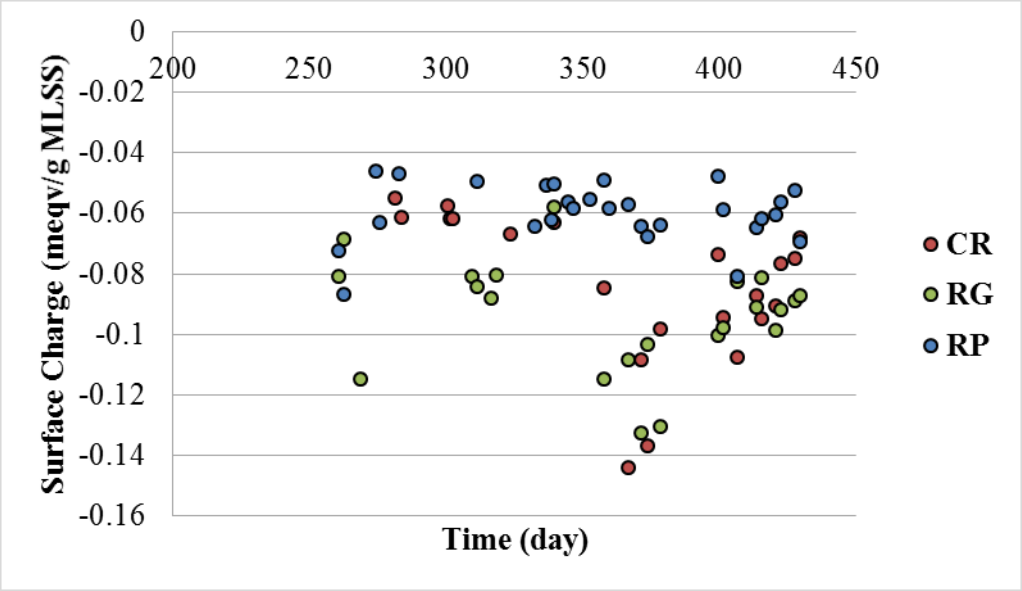

Figure 4.79. Surface charges of sludges taken from reactors CR, RG and RP.

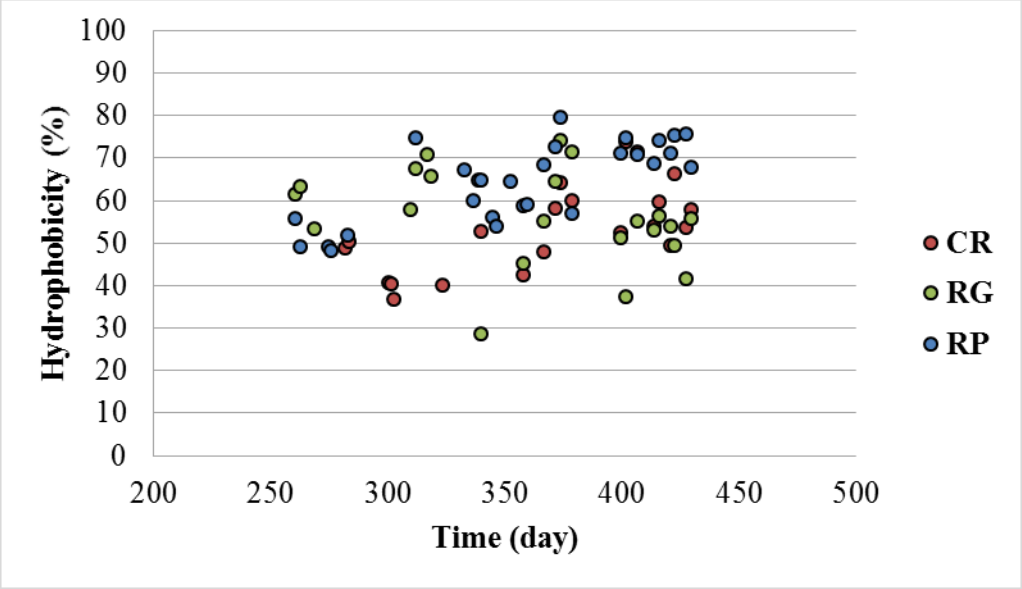

Figure 4.80. Hydrophobicities of sludges taken from reactors CR, RG and RP.

According to Figure 4.80., the hydrophobicities of CR and RG were close to each other, while RP had a higher hydrophobicity. Average hydrophobicities of CR, RG and RP were found as 53%±10, 56%±11 and 65%±9, respectively. Additionally, t-test was applied for the hydrophobicities of CR, RG and RP. Results are given in Table 4.12. This table indicates that CR and RG sludges had close hydrophobicities, because the p value of the t-test was

higher than 0.05. On the other hand, RG and RP as well as CR and RP were different from each other as seen from very low p values.

Table 4.12. Results of t-test for hydrophobicity.

Comparison of Reactors	p value
CR-RG	0.299737
CR-RP	0.000180
RG-RP	0.00010

As mentioned before, the hydrophobic fraction is made up of proteins. Mostly amino acids contribute to the hydrophobicity in the EPS structure (Durmaz and Sanin, 2003). RP sludge was fed with peptone only, so it had the highest hydrophobicity. On the other hand, CR and RG sludges showed a similar trend in terms of hydrophobicity as in the case of surface charge. This indicates that they had somehow similar physical characteristics.

As mentioned before, there is a negative correlation between surface charge and hydrophobicity. The sludges which have high surface charges, are less hydrophobic (Liao et al., 2001). The results of present experiments showed that CR and RG had higher negative surface charges and lower hydrophobicies. Also, RP sludge had a lower surface charge, and a more hydrophobic character.

4.4. Sorption Tests

As mentioned before, sorption tests were done in order to determine the sorption capacity of silver on different activated sludge. For these tests, sludges taken from CR, RG and RP reactors were used. The details of tests are presented in Materials and Methods. Linear and Freundlich isotherms were plotted for CR, RG and RP sludges (presented in **Appendix C).** The isotherms belonging to Ag sorption show that neither the linear nor the Freundlich isotherms had a high regression coefficient. As mentioned earlier, these tests were designed to be used in MINTEQA2 program within the scope of the project.

Additionally, the soluble part of Ag after 1 hour shaking was also calculated by using the results of these sorption tests. Results showed that:

- For CR sludge, soluble part in Ag varied between 3 and 8 %
- For RG sludge, soluble part in Ag varied between 4 and 11 % (average of two tests)
- For RP sludge, soluble part in Ag varied between 2 and 4 % (average of two tests)

According to the results it can be said that, a large portion of Ag partitioned into solid phase. It was observed that 2-10 % of Ag remained in the soluble part at the end of 1 hour.

5. CONCLUSION

The main objective of this thesis study was to investigate the inhibitory effect of silver on different types of activated sludges. For this purpose, three synthetic wastewaters with a different organic composition were prepared. Then, three laboratory-scale activated sludge reactors (CR, RG and RP) were fed at the same COD/TKN ratio of 10. In addition, differences between the surface charge and hydrophobicity characteristics of these sludges were determined.

In order to differentiate carbonaceous oxygen uptake ($C\text{-}O_2$) from the nitrogenous oxygen uptake ($N\text{-}O_2$), ATU was used as a nitrification inhibitor in respirometry tests. The results of these tests showed that heterotrophic activity in CR, RG and RP reactors were higher than autotrophic activity as expected. At the C/N ratio of 10 heterotrophic bacteria were more dominant compared to nitrifying bacteria.

The effect of Ag on these activated sludges was examined by using respirometry. The results showed that CR and RG sludges were affected by the addition of Ag. After 3 mg/L Ag concentration, these sludges were highly inhibited. These results are in accordance with the results of the former study (Ayyıldız, 2013). According to that study, R1 sludge, which had a C/N ratio of 10, was slightly affected at 3 mg/L Ag and completely affected at 5 mg/L Ag concentration. However, RP sludge performance was not affected by Ag addition. It was observed that this sludge could tolerate Ag up to 5 mg/L. According to results, it was thought that RP sludge was not affected by Ag because of the composition of the feed. For this reason, two respirometry tests were done by using Feed G and Feed P with RG and RP sludges. The results showed that sludges fed with Feed P was not affected by Ag addition. This indicated that protein in peptone formed complexes with Ag and reduced its inhibitory effect on sludge.

The second aim of this study was to determine the differences between surface charge and hydrophobicites of CR, RG and RP sludges. CR and RG sludges had higher surface charges than RP sludge. The feeds of CR and RG reactors contained glucose which resulted in higher surface charges than peptone. On the other hand, RP sludge had the highest

hydrophobicity, while CR and RG sludges had lower hydrophobicities which were close to each other. Since RP sludge was fed with peptone only containing mainly protein, the protein amount in the EPS of the sludge led to higher hydrophobicity.

The results of this study showed that feed composition had an important role, not only on the physical properties of a sludge, but also on the inhibitory effect of silver. A sludge fed with peptone only as an organic substrate may tolerate higher silver concentrations compared to a sludge fed with a mixture of acetate, glucose and peptone or only glucose. This indicates that the effect of silver in real biological treatment systems may change due to the changes in the composition of influent wastewater.

The respirometric tests alone are not sufficient for assessing the relationship between feed composition and silver inhibition. Factors such as composition of microbial products and metal speciation play also an important role. Therefore, for proper comparison, respirometric data and analytic measurements should be supported with EPS characterization.

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APPENDIX A: MONITORING OF ACTIVATED SLUDGE REACTORS

Phase 1 – OPERATION OF REACTORS AT DIFFERENT COD/TKN (C/N) RATIOS

Reactor 1 (R1): COD/TKN = 10, Reactor 2 (R2): COD/TKN = 5 and Reactor 3 (R3): COD/TKN = 0

Table A.1. Operational results of R1 – July 2013.

Run	**1**		**2**		**3**		**4**		**5**	
Date	**1.7.2013**	**3.7.2013**	**8.7.2013**	**10.7.2013**	**15.7.2013**	**17.7.2013**	**22.7.2013**	**24.7.2013**	**29.7.2013**	**31.7.2013**
pH	8.01	8.62	8.09	8.86	8.59	8.59	8.02	8.66	8.63	8.85
Temperature (^{o}C)	24.2	24.3	24.2	24	24.2	24.3	22.8	24.6	25.3	26
COD (mg/L)	941	144	858	219	538	130	936	122	739	66
COD removal (%)	84.7		74.5		75.8		87.0		91.1	
MLSS (mg/L)	4200		4010		4345		3350		2675	
MLVSS (mg/L)	3185		2950		3375		2460		2050	
Run period (day)	1.92		1.89		1.76		1.93		1.76	
F:M ratio (mg COD/mg MLVSS.day)	0.15		0.15		0.09		0.20		0.20	
S_0/X_0 (mg COD/mg MLVSS)	0.30		0.29		0.16		0.38		0.36	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.13		0.11		0.07		0.17		0.19	

Table A.2. Operational results of R1 – August 2013.

Run	**1**		**2**		**3**		**4**	
Date	**5.8.2013**	**7.8.2013**	**12.8.2013**	**14.8.2013**	**19.8.2013**	**21.8.2013**	**26.8.2013**	**28.8.2013**
pH	8.27	8.98	8.53	8.72	7.7	9.01	8.18	9.36
Temperature (^{o}C)	25	26	26.2	26.6	26.1	26.6	25.6	26.3
COD (mg/L)	947	74	1081	87	1100	84	962	97
COD removal (%)	92.2		92.0		92.4		89.9	
MLSS (mg/L)	2795		2625		2240		2370	
MLVSS (mg/L)	2105		2105		1730		1780	
Run period (day)	1.94		1.80		1.95		1.94	
F:M ratio (mg COD/mg MLVSS.day)	0.23		0.29		0.33		0.28	
S_0/X_0 (mg COD/mg MLVSS)	0.45		0.51		0.64		0.54	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.21		0.26		0.30		0.25	

Table A.3. Operational results of R1 – September 2013.

Run	**1**		**2**		**3**		**4**		**5**	
Date	**2.9.2013**	**4.9.2013**	**9.9.2013**	**11.9.2013**	**16.9.2013**	**18.9.2013**	**23.9.2013**	**25.9.2013**	**30.9.2013**	**2.10.2013**
pH	8.03	8.8	8.14	9.35	9.24	9.32	8.42	8.82	7.61	7.71
Temperature (°C)	23.6	23.8	21.6	23.4	23.3	23.7	20.4	21.8	21.5	19.6
COD (mg/L)	1067	258	995	91	1060	247	940	321	1123	342
COD removal (%)	75.8		90.9		76.7		65.9		69.5	
MLSS (mg/L)	2525		1860		3205		3230		4425	
MLVSS (mg/L)	1890		1410		2100		2300		3225	
Run period (day)	1.94		1.87		1.96		1.94		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.29		0.38		0.26		0.21		0.18	
S_0/X_0 (mg COD/mg MLVSS)	0.56		0.71		0.50		0.41		0.35	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.22		0.34		0.20		0.14		0.12	

Table A.4. Operational results of R1 – October 2013.

Run	**1**		**2**		**3**	
Date	**7.10.2013**	**9.10.2013**	**21.10.2013**	**23.10.2013**	**28.10.2013**	**30.10.2013**
pH	6.47	7.06	6.92	6.64	6.74	6.22
Temperature (°C)	20.1	25.6	20.1	19.7	20.2	20.4
COD (mg/L)	1074	157	1040	320	1038	164
COD removal (%)	85.4		69.2		84.2	
MLSS (mg/L)	3880		3940		3835	
MLVSS (mg/L)	3065		2800		2950	
Run period (day)	1.77		2.01		1.99	
F:M ratio (mg COD/mg MLVSS.day)	0.20		0.18		0.18	
S_0/X_0 (mg COD/mg MLVSS)	0.35		0.37		0.35	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.17		0.13		0.15	

Table A.5. Operational results of R1 – November 2013.

Run	**1**		**2**		**3**		**4**	
Date	**4.11.2013**	**6.11.2013**	**11.11.2013**	**13.11.2013**	**18.11.2013**	**20.11.2013**	**25.11.2013**	**27.11.2013**
pH	6.53	5.99	5.97	5.98	5.98	5.97	6.35	5.82
Temperature (^{o}C)	23.1	26.9	25.5	26	21.9	24.7	26.8	26
COD (mg/L)	1018	81	953	70	1029	89	1091	134
COD removal (%)	92.0		92.7		91.4		87.7	
MLSS (mg/L)	2615		2510		3370		4710	
MLVSS (mg/L)	2075		1950		2615		3575	
Run period (day)	1.92		1.96		1.95		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.26		0.25		0.20		0.16	
S_0/X_0 (mg COD/mg MLVSS)	0.49		0.49		0.39		0.31	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.24		0.23		0.18		0.14	

Table A.6. Operational results of R1 – December 2013.

Run	**1**		**2**		**3**		**4**	
Date	**2.12.2013**	**4.12.2013**	**9.12.2013**	**11.12.2013**	**16.12.2013**	**18.12.2013**	**23.12.2013**	**25.12.2013**
pH	6.31	5.77	6.47	6	6.17	5.8	5.89	5.75
Temperature (^{o}C)	25.3	24.2	18.8	26.2	25.3	25.9	25.1	31
COD (mg/L)	1098	42	1008	18	977	57	975	56
COD removal (%)	96.2		98.2		94.2		94.3	
MLSS (mg/L)	5225		3350		3845		4345	
MLVSS (mg/L)	3955		2635		2940		3270	
Run period (day)	1.95		1.98		1.96		1.94	
F:M ratio (mg COD/mg MLVSS.day)	0.14		0.19		0.17		0.15	
S_0/X_0 (mg COD/mg MLVSS)	0.28		0.38		0.33		0.30	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.14		0.19		0.16		0.14	

Table A.7. Operational results of R1 – January 2014.

Run	**1**		**2**		**3**		**4**	
Date	**6.1.2014**	**8.1.2014**	**13.1.2014**	**15.1.2014**	**20.1.2014**	**22.1.2014**	**27.1.2014**	**29.1.2014**
pH	6.05	5.66	6.98	6.09	6.22	5.81	6.78	5.78
Temperature (^{o}C)	24.2	31.8	19.2	32	26.7	32.4	25.7	32.8
COD (mg/L)	909	36	970	32	936	27	1030	54
COD removal (%)	96.0		96.7		97.1		94.8	
MLSS (mg/L)	3540		3510		4230		3675	
MLVSS (mg/L)	2780		2455		3280		2875	
Run period (day)	1.97		1.93		1.96		1.95	
F:M ratio (mg COD/mg MLVSS.day)	0.17		0.20		0.15		0.18	
S_0/X_0 (mg COD/mg MLVSS)	0.33		0.40		0.29		0.36	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.16		0.20		0.14		0.17	

Table A.8. Operational results of R1 – February 2014.

Run	**1**		**2**		**3**		**4**	
Date	**3.2.2014**	**5.2.2014**	**10.2.2014**	**12.2.2014**	**17.2.2014**	**19.2.2014**	**24.2.2014**	**26.2.2014**
pH	6.55	6.23	6.86	6.04	6.8	6.77	6.32	6.64
Temperature (^{o}C)	23.9	29.6	26.3	33.9	20.5	28.5	25.7	27.2
COD (mg/L)	973	62	1030	62	982	46	998	110
COD removal (%)	93.6		94.0		95.3		89.0	
MLSS (mg/L)	3215		3305		3125		3240	
MLVSS (mg/L)	2655		2740		2600		2540	
Run period (day)	1.95		1.95		1.94		1.78	
F:M ratio (mg COD/mg MLVSS.day)	0.19		0.19		0.19		0.22	
S_0/X_0 (mg COD/mg MLVSS)	0.37		0.38		0.38		0.39	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.18		0.18		0.19		0.20	

Table A.9. Operational results of R1 – March 2014.

Run	**1**		**2**		**3**		**4**	
Date	**3.3.2014**	**5.3.2014**	**10.3.2014**	**12.3.2014**	**24.3.2014**	**26.3.2014**	**31.3.2014**	**2.4.2014**
pH	7.49	8.63	7.58	8.83	7.64	8.17	8.08	8.49
Temperature (^{o}C)	25.1	28.2	23	24.5	24.4	27.5	25.3	24.8
COD (mg/L)	1003	86	970	99	924	102	818	76
COD removal (%)	91.4		89.8		89.0		90.7	
MLSS (mg/L)	3380		3715		3510		4365	
MLVSS (mg/L)	2460		2580		2485		3050	
Run period (day)	1.78		1.78		1.80		1.80	
F:M ratio (mg COD/mg MLVSS.day)	0.23		0.21		0.21		0.15	
S_0/X_0 (mg COD/mg MLVSS)	0.41		0.38		0.37		0.27	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.21		0.19		0.18		0.14	
	26.3.2014				**28.3.2014**			
Surface charge (meqv/g MLSS)	-0.024				-0.022			
Hydrophobicity (%)	59				65			

Table A.10. Operational results of R1 – April 2014.

Run	**1**		**2**		**3**	
Date	**7.4.2014**	**9.4.2014**	**14.4.2014**	**16.4.2014**	**28.4.2014**	**30.4.2014**
pH	7.76	8.52	7.95	8.42	7.68	7.65
Temperature (^{o}C)	24	23.6	26.2	27.5	20.5	22.2
COD (mg/L)	885	92	991	96	925	228
COD removal (%)	89.6		90.3		75.4	
MLSS (mg/L)	3975		3445		4150	
MLVSS (mg/L)	2795		2480		3030	
Run period (day)	1.80		1.79		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.18		0.22		0.16	
S_0/X_0 (mg COD/mg MLVSS)	0.32		0.40		0.31	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.16		0.20		0.12	

Table A.11. Operational results of R1 – May 2014.

Run	**1**		**2**		**3**	
Date	**5.5.2014**	**7.5.2014**	**12.5.2014**	**14.5.2014**	**26.5.2014**	**28.5.2014**
pH	7.82	8.22	8.58	8.23	8.58	8.12
Temperature (^{o}C)	22.5	22.2	21.4	22.1	22.8	25.5
COD (mg/L)	984	42	910	69	979	49
COD removal (%)	95.7		92.4		95.0	
MLSS (mg/L)	3760		4280		5130	
MLVSS (mg/L)	2580		2765		3100	
Run period (day)	1.78		1.80		2.15	
F:M ratio (mg COD/mg MLVSS.day)	0.21		0.18		0.15	
S_0/X_0 (mg COD/mg MLVSS)	0.38		0.33		0.32	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.21		0.17		0.14	

Table A.12. Operational results of R1 – June 2014.

Run	**1**		**2**		**3**		**4**	
Date	**2.6.2014**	**4.6.2014**	**10.6.2014**	**12.6.2014**	**16.6.2014**	**18.6.2014**	**23.6.2014**	**25.6.2014**
pH	8.42	8.08	7.67	7.82	7.86	8.56	7.53	8.54
Temperature (^{o}C)	21.6	21.9	21.7	22.9	24.2	25.5	22.2	24.1
COD (mg/L)	905	95	988	134	938	88	945	91
COD removal (%)	89.5		86.4		90.6		90.4	
MLSS (mg/L)	4325		4300		4215		3645	
MLVSS (mg/L)	2640		3150		3200		2430	
Run period (day)	1.97		1.96		1.97		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.17		0.16		0.15		0.20	
S_0/X_0 (mg COD/mg MLVSS)	0.34		0.31		0.29		0.39	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.16		0.14		0.13		0.18	

Table A.13. Operational results of R1 – July 2014.

Run	**1**		**2**		**3**	
Date	**30.6.2014**	**2.7.2014**	**7.7.2014**	**9.7.2014**	**14.7.2014**	**16.7.2014**
pH	7.51	7.84	6.9	7.15	7.11	6.54
Temperature (°C)	20.4	24.1	23.5	25.1	25.6	25
COD (mg/L)	1011	53	944	85	946	48
COD removal (%)	94.8		91.0		94.9	
MLSS (mg/L)	3665		3945		3725	
MLVSS (mg/L)	2960		3110		2810	
Run period (day)	1.95		1.97		1.97	
F:M ratio (mg COD/mg MLVSS.day)	0.18		0.15		0.17	
S_0/X_0 (mg COD/mg MLVSS)	0.34		0.30		0.34	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.17		0.14		0.16	

Table A.14. Operational results of R1 – August 2014.

Run	**1**		**2**		**3**		**4**	
Date	**4.8.2014**	**6.8.2014**	**11.8.2014**	**13.8.2014**	**18.8.2014**	**20.8.2014**	**25.8.2014**	**27.8.2014**
pH	6.38	7.03	7.56	8.04	7.04	7.99	7.75	8.26
Temperature (°C)	26.1	27.6	22.6	27.1	25.3	26.5	24.7	26.5
COD (mg/L)	949	24	1000	30	1091	27	911	62
COD removal (%)	97.5		97.0		97.5		93.2	
MLSS (mg/L)	2585		4450		4510		5070	
MLVSS (mg/L)	2135		3435		3510		3865	
Run period (day)	1.95		1.94		1.95		1.97	
F:M ratio (mg COD/mg MLVSS.day)	0.23		0.15		0.16		0.12	
S_0/X_0 (mg COD/mg MLVSS)	0.44		0.29		0.31		0.24	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.22		0.15		0.16		0.11	

Table A.15. Operational results of R1 – September 2014.

Run	**1**		**2**	
Date	**1.9.2014**	**3.9.2014**	**8.9.2014**	**10.9.2014**
pH	7.42	6.97	7.66	8.03
Temperature (^{o}C)	24.3	25.1	24.6	25
COD (mg/L)	965	88	869	24
COD removal (%)	90.9		97.2	
MLSS (mg/L)	5270		4265	
MLVSS (mg/L)	3815		3225	
Run period (day)	1.96		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.13		0.14	
S_0/X_0 (mg COD/mg MLVSS)	0.25		0.27	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.12		0.13	

Table A.16. Operational results of R2 – July 2013.

Run	**1**		**2**		**3**		**4**		**5**	
Date	**1.7.2013**	**3.7.2013**	**8.7.2013**	**10.7.2013**	**15.7.2013**	**17.7.2013**	**22.7.2013**	**24.7.2013**	**29.7.2013**	**31.7.2013**
pH	8.58	8.54	8.24	8.96	7.76	8.65	8.58	8.62	7.91	8.66
Temperature (^{o}C)	22.9	23.9	22.9	23.8	23.1	24	22	24.3	24.2	27.2
COD (mg/L)	454	35	386	58	418	44	509	32	414	67
COD removal (%)	92.3		85.0		89.5		93.7		83.8	
NH_4-N (mg/L)	61.5	0	57	0	53.5	0	61.5	0	60.5	0
NH_4 -N removal (%)	100.0		100.0		100.0		100.0		100.0	
MLSS (mg/L)	3295		3890		3315		3695		4055	
MLVSS (mg/L)	2230		2550		2240		2445		2600	
Run period (day)	1.93		1.95		1.76		1.94		1.77	
F:M ratio (mg COD/mg MLVSS.day)	0.11		0.08		0.11		0.11		0.09	
S_0/X_0 (mg COD/mg MLVSS)	0.20		0.15		0.19		0.21		0.16	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.10		0.07		0.09		0.10		0.08	

Table A.17. Operational results of R2 – August 2013.

Run	**1**		**2**		**3**		**4**	
Date	**5.8.2013**	**7.8.2013**	**12.8.2013**	**14.8.2013**	**19.8.2013**	**21.8.2013**	**26.8.2013**	**28.8.2013**
pH	7.91	8.76	7.6	7.94	8.25	8.78	7.78	8.48
Temperature (°C)	24.2	25.8	25.3	26.4	24.8	26.1	24.1	26.3
COD (mg/L)	447	19	373	24	457	20	429	15
COD removal (%)	95.7		93.6		95.6		96.5	
NH_4-N (mg/L)	61.5	0	55	0	53.5	0		
NH_4 -N removal (%)	100.0		100.0		100.0			
MLSS (mg/L)	4485		4600		4885		4095	
MLVSS (mg/L)	2835		2995		3150		2575	
Run period (day)	1.95		1.80		1.96		1.95	
F:M ratio (mg COD/mg MLVSS.day)	0.08		0.07		0.07		0.09	
S_0/X_0 (mg COD/mg MLVSS)	0.16		0.12		0.15		0.17	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.08		0.06		0.07		0.08	

Table A.18. Operational results of R2 – September 2013.

Run	**1**		**2**		**3**		**4**		**5**	
Date	**2.9.2013**	**4.9.2013**	**9.9.2013**	**11.9.2013**	**16.9.2013**	**18.9.2013**	**23.9.2013**	**25.9.2013**	**30.9.2013**	**2.10.2013**
pH	7.87	8.73	7.86	8.09	7.95	8.5	7.84	8.32	7.92	8.55
Temperature (°C)	22.3	22.9	22.3	22.7	21.6	23.9	19.8	21.4	22.3	20.4
COD (mg/L)	458	12	218	6	428	10	502	20	436	28
COD removal (%)	97.4		97.2		97.7		96.0		93.6	
MLSS (mg/L)	3615		3450		3945		4520		4830	
MLVSS (mg/L)	2290		2295		2590		2835		2980	
Run period (day)	1.95		1.87		1.97		1.95		1.97	
F:M ratio (mg COD/mg MLVSS.day)	0.10		0.05		0.08		0.09		0.07	
S_0/X_0 (mg COD/mg MLVSS)	0.20		0.09		0.17		0.18		0.15	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.10		0.05		0.08		0.09		0.07	

Table A.19. Operational results of R2 – October 2013.

Run	**1**		**2**		**3**	
Date	**7.10.2013**	**9.10.2013**	**21.10.2013**	**23.10.2013**	**28.10.2013**	**30.10.2013**
pH	8.03	8.21	7.69	8.1	7.74	8.4
Temperature (°C)	19.5	28.6	21.2	24.6	23.2	28.2
COD (mg/L)	442	30	451	48	417	40
COD removal (%)	93.2		89.4		90.4	
MLSS (mg/L)	3905		3795		2825	
MLVSS (mg/L)	2655		2260		1665	
Run period (day)	1.80		2.02		2.00	
F:M ratio (mg COD/mg MLVSS.day)	0.09		0.10		0.13	
S_0/X_0 (mg COD/mg MLVSS)	0.17		0.20		0.25	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.09		0.09		0.11	

Table A.20. Operational results of R2 – November 2013.

Run	**1**		**2**		**3**		**4**	
Date	**4.11.2013**	**6.11.2013**	**11.11.2013**	**13.11.2013**	**18.11.2013**	**20.11.2013**	**25.11.2013**	**27.11.2013**
pH	8.29	8.31	7.59	8.51	7.81	8.66	8.2	8.63
Temperature (°C)	21.9	27.8	24.3	26.5	21.3	26.9	23.6	22.6
COD (mg/L)	507	23	473	27	472	42	524	55
COD removal (%)	95.5		94.3		91.1		89.5	
MLSS (mg/L)	2745		3030		2910		4095	
MLVSS (mg/L)	1730		1790		1865		2350	
Run period (day)	1.92		1.96		1.96		1.97	
F:M ratio (mg COD/mg MLVSS.day)	0.15		0.13		0.13		0.11	
S_0/X_0 (mg COD/mg MLVSS)	0.29		0.26		0.25		0.22	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.15		0.13		0.12		0.10	

Table A.21. Operational results of R2 – December 2013.

Run	**1**		**2**		**3**		**4**	
Date	**2.12.2013**	**4.12.2013**	**9.12.2013**	**11.12.2013**	**16.12.2013**	**18.12.2013**	**23.12.2013**	**25.12.2013**
pH	7.81	8.78	7.38	8.09	7.64	8.5	8.87	8.67
Temperature (^{o}C)	24.8	26.6	18.7	27.5	24.5	27.6	23.4	24.9
COD (mg/L)	527	62	486	19	484	17	510	20
COD removal (%)	88.2		96.1		96.5		96.1	
MLSS (mg/L)	3620		3545		3910		4000	
MLVSS (mg/L)	2115		2040		2190		2245	
Run period (day)	1.95		1.98		1.97		1.94	
F:M ratio (mg COD/mg MLVSS.day)	0.13		0.12		0.11		0.12	
S_0/X_0 (mg COD/mg MLVSS)	0.25		0.24		0.22		0.23	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.11		0.12		0.11		0.11	

Table A.22. Operational results of R2 – January 2014.

Run	**1**		**2**		**3**		**4**	
Date	**6.1.2014**	**8.1.2014**	**13.1.2014**	**15.1.2014**	**20.1.2014**	**22.1.2014**	**27.1.2014**	**29.1.2014**
pH	7.93	8.52	7.67	8.15	7.87	8.53	7.72	8.13
Temperature (^{o}C)	25.3	26.3	18.3	26.1	24.8	23.6	24.7	25.4
COD (mg/L)	447	49	410	15	452	22	479	24
COD removal (%)	89.0		96.3		95.1		95.0	
MLSS (mg/L)	4230		3995		4200		4130	
MLVSS (mg/L)	2360		1850		2360		2280	
Run period (day)	1.98		1.93		1.96		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.10		0.11		0.10		0.11	
S_0/X_0 (mg COD/mg MLVSS)	0.19		0.22		0.19		0.21	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.09		0.11		0.09		0.10	

Table A.23. Operational results of R2 – February 2014.

Run	**1**		**2**		**3**		**4**	
Date	**3.2.2014**	**5.2.2014**	**10.2.2014**	**12.2.2014**	**17.2.2014**	**19.2.2014**	**24.2.2014**	**26.2.2014**
pH	7.8	8.5	8.35	8.42	7.54	8.14	7.85	8.31
Temperature (^{o}C)	24	23.5	24.7	24.7	19.5	25	24.4	24.2
COD (mg/L)	453	25	444	79	487	66	487	53
COD removal (%)	94.5		82.2		86.4		89.1	
MLSS (mg/L)	4470		4625		4490		4765	
MLVSS (mg/L)	2470		2590		2585		2715	
Run period (day)	1.95		1.96		1.95		1.79	
F:M ratio (mg COD/mg MLVSS.day)	0.09		0.09		0.10		0.10	
S_0/X_0 (mg COD/mg MLVSS)	0.18		0.17		0.19		0.18	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.09		0.07		0.08		0.09	

Table A.24. Operational results of R2 – March 2014.

Run	**1**		**2**		**3**		**4**	
Date	**3.3.2014**	**5.3.2014**	**10.3.2014**	**12.3.2014**	**24.3.2014**	**26.3.2014**	**31.3.2014**	**2.4.2014**
pH	7.83	8.45	8.67	9.23	8.86	9.16	9.49	9.22
Temperature (^{o}C)	25.5	24.9	23.3	24.6	24.3	25.4	20.6	22.8
COD (mg/L)	505	21	460	32	413	29	470	68
COD removal (%)	95.8		93.0		93.0		85.5	
MLSS (mg/L)	5070		4250		4720		4780	
MLVSS (mg/L)	2750		2320		2550		2590	
Run period (day)	1.78		1.79		1.80		1.80	
F:M ratio (mg COD/mg MLVSS.day)	0.10		0.11		0.09		0.10	
S_0/X_0 (mg COD/mg MLVSS)	0.18		0.20		0.16		0.18	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.10		0.10		0.08		0.09	
	26.3.2014				**28.3.2014**			
Surface charge (meqv/g MLSS)	-0.046				-0.051			
Hydrophobicity (%)	60				66			

Table A.25. Operational results of R2 – April 2014.

Run	**1**		**2**		**3**	
Date	**7.4.2014**	**9.4.2014**	**14.4.2014**	**16.4.2014**	**28.4.2014**	**30.4.2014**
pH	8.68	9.15	8.22	8.93	6.75	6.05
Temperature (^{o}C)	25	21.6	24.3	24.8	19.6	21.4
COD (mg/L)	464	153	455	95	451	61
COD removal (%)	67.0		79.1		86.5	
MLSS (mg/L)	5275		4870		3585	
MLVSS (mg/L)	2885		2600		2260	
Run period (day)	1.80		1.79		1.97	
F:M ratio (mg COD/mg MLVSS.day)	0.09		0.10		0.10	
S_0/X_0 (mg COD/mg MLVSS)	0.16		0.18		0.20	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.06		0.08		0.09	
	10.4.2014					
Surface charge (meqv/g MLSS)	-0.048					
Hydrophobicity (%)	56					

Table A.26. Operational results of R2 – May 2014.

Run	**1**		**2**		**3**	
Date	**5.5.2014**	**7.5.2014**	**12.5.2014**	**14.5.2014**	**26.5.2014**	**28.5.2014**
pH	7.13	6.99	7.5	7.89	7.64	7.93
Temperature (^{o}C)	20.8	21	20.2	22.1	19.5	24.9
COD (mg/L)	437	53	446	49	395	12
COD removal (%)	87.9		89.0		97.0	
MLSS (mg/L)	4860		4700		4980	
MLVSS (mg/L)	2550		2880		3105	
Run period (day)	1.79		1.81		2.15	
F:M ratio (mg COD/mg MLVSS.day)	0.10		0.09		0.06	
S_0/X_0 (mg COD/mg MLVSS)	0.17		0.15		0.13	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.08		0.08		0.06	

Table A.27. Operational results of R2 – June 2014.

Run	**1**		**2**		**3**		**4**	
Date	**2.6.2014**	**4.6.2014**	**10.6.2014**	**12.6.2014**	**16.6.2014**	**18.6.2014**	**23.6.2014**	**25.6.2014**
pH	8.65	8.19	7.86	8.64	8.39	8.62	8.34	8.74
Temperature (^{o}C)	21.4	21.7	20.8	23	23.8	25.2	21.7	24.1
COD (mg/L)	395	22	460	12	430	54	465	25
COD removal (%)	94.4		97.4		87.4		94.6	
MLSS (mg/L)	4950		4865		5315		5015	
MLVSS (mg/L)	3000		3175		3410		2890	
Run period (day)	1.98		1.97		1.97		1.97	
F:M ratio (mg COD/mg MLVSS.day)	0.07		0.07		0.06		0.08	
S_0/X_0 (mg COD/mg MLVSS)	0.13		0.14		0.13		0.16	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.06		0.07		0.06		0.08	

Table A.28. Operational results of R2 – July 2014.

Run	**1**		**2**		**3**	
Date	**30.6.2014**	**2.7.2014**	**7.7.2014**	**9.7.2014**	**14.7.2014**	**16.7.2014**
pH	8.34	8.24	7.87	8.5	8.34	8.52
Temperature (^{o}C)	19.8	24.1	22.9	25.3	25.2	26
COD (mg/L)	475	46	389	22	343	61
COD removal (%)	90.3		94.3		82.2	
MLSS (mg/L)	5900		5135		5025	
MLVSS (mg/L)	3640		3445		3155	
Run period (day)	1.95		1.98		1.98	
F:M ratio (mg COD/mg MLVSS.day)	0.07		0.06		0.05	
S_0/X_0 (mg COD/mg MLVSS)	0.13		0.11		0.11	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.06		0.05		0.05	

Table A.29. Operational results of R2 – August 2014.

Run	**1**		**2**		**3**		**4**	
Date	**4.8.2014**	**6.8.2014**	**11.8.2014**	**13.8.2014**	**18.8.2014**	**20.8.2014**	**25.8.2014**	**27.8.2014**
pH	7.66	8.2	7.68	8.15	8.11	8.58	8.21	8.7
Temperature (^{o}C)	25.8	27.5	21.7	26.8	24.7	26.2	24	26.3
COD (mg/L)	409	37	413	46	439	31	410	36
COD removal (%)	91.0		88.9		92.9		91.2	
MLSS (mg/L)	6115		5815		5095		6085	
MLVSS (mg/L)	4050		3485		3075		3710	
Run period (day)	1.95		1.95		1.98		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.05		0.06		0.07		0.06	
S_0/X_0 (mg COD/mg MLVSS)	0.10		0.12		0.14		0.11	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.05		0.05		0.07		0.05	

Table A.30. Operational results of R2 – September 2014.

Run	**1**		**2**	
Date	**1.9.2014**	**3.9.2014**	**8.9.2014**	**10.9.2014**
pH	8.28	8.67	8.11	8.33
Temperature (^{o}C)	23.4	24.8	23.4	24.4
COD (mg/L)	430	176	442	23
COD removal (%)	59.1		94.8	
MLSS (mg/L)	6135		3725	
MLVSS (mg/L)	3450		2310	
Run period (day)	1.96		1.97	
F:M ratio (mg COD/mg MLVSS.day)	0.06		0.10	
S_0/X_0 (mg COD/mg MLVSS)	0.12		0.19	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.04		0.09	

Table A.31. Operational results of R3 – July 2013.

Run	**1**		**2**		**3**		**4**		**5**	
Date	**1.7.2013**	**2.7.2013**	**8.7.2013**	**9.7.2013**	**15.7.2013**	**16.7.2013**	**22.7.2013**	**23.7.2013**	**29.7.2013**	**30.7.2013**
pH	7.29	6.15	7.43	6.14	7.59	6.32	7.58	6.12	7.69	6.16
Temperature (^{o}C)	24.7	23.9	24.8	25	24.7	24.8	24.1	23.9	26.5	26.7
NH_4-N (mg/L)	240	35.75	235	22.5	205	17.5	320	86	315	89
NH_4 -N removal (%)	85.1		90.4		91.5		73.1		71.7	
NH_3 (mg/L)	2.7	0.0	3.6	0.0	4.5	0.0	6.6	0.1	9.8	0.1
MLSS (mg/L)	2430		2520		2135		2185		2465	
MLVSS (mg/L)	870		900		795		745		935	
Run period (day)	1.03		1.04		0.80		1.03		0.76	
S_0/X_0 (mg NH_4 -N/mg MLVSS)	0.28		0.26		0.26		0.43		0.34	
$q_{specific}$ (mg NH_4 -N/mg MLVSS.day)	0.23		0.23		0.29		0.30		0.32	

Table A.32. Operational results of R3 – August 2013.

Run	**1**		**2**		**3**		**4**	
Date	**5.8.2013**	**6.8.2013**	**12.8.2013**	**13.8.2013**	**19.8.2013**	**21.8.2013**	**26.8.2013**	**28.8.2013**
pH	7.44	6.12	7.64	6.57	7.7	6.64	7.54	6.59
Temperature (^{o}C)	26.2	25.9	26.9	26.8	27	26.1	26.6	26.2
NH_4-N (mg/L)	235	27	227.3	17	460	56.5	-	-
NH_4 -N removal (%)	88.5		92.5		87.7		-	
NH_3 (mg/L)	4.1	0.0	6.5	0.0	15.1	0.2	-	-
MLSS (mg/L)	2565		3065		4660		4315	
MLVSS (mg/L)	1010		1115		1420		1225	
Run period (day)	1.04		0.81		1.94		1.92	
S_0/X_0 (mg NH_4 -N/mg MLVSS)	0.23		0.20		0.32		-	
$q_{specific}$ (mg NH_4 -N/mg MLVSS.day)	0.20		0.23		0.15		-	

Table A.33. Operational results of R3 – September 2013.

Run	**1**		**2**		**3**		**4**		**5**	
Date	**2.9.2013**	**4.9.2013**	**9.9.2013**	**11.9.2013**	**16.9.2013**	**18.9.2013**	**23.9.2013**	**25.9.2013**	**30.9.2013**	**2.10.2013**
pH	7.68	6.73	7.59	6.52	7.74	6.9	7.83	6.83	7.63	6.55
Temperature (^{o}C)	25.1	23.6	22.8	23.3	23.8	24.5	22.2	22	22.8	20.4
NH_4-N (mg/L)	465	57	467.5	62.5	470	88	445	56	470	66
NH_4 -N removal (%)	87.7		86.6		81.3		87.4		86.0	
NH_3 (mg/L)	12.8	0.2	8.9	0.1	13.5	0.4	14.0	0.2	9.8	0.1
MLSS (mg/L)	4145		3380		3690		3095		4550	
MLVSS (mg/L)	1210		1120		1020		925		1405	
Run period (day)	1.93		1.86		1.95		1.93		1.95	
S_0/X_0 (mg NH_4 -N/mg MLVSS)	0.38		0.42		0.46		0.48		0.33	
$q_{specific}$ (mg NH_4 -N/mg MLVSS.day)	0.17		0.19		0.19		0.22		0.15	

Table A.34. Operational results of R3 – October 2013.

Run	**1**		**2**		**3**	
Date	**7.10.2013**	**9.10.2013**	**21.10.2013**	**23.10.2013**	**28.10.2013**	**30.10.2013**
pH	7.52	6.65	7.45	6.45	7.59	6.96
Temperature (^{o}C)	21.4	22.8	21.2	24.5	21.9	22.8
NH_4-N (mg/L)	460	52.5	505	74	467.5	66
NH_4 -N removal (%)	88.6		85.3		85.9	
NH_3 (mg/L)	6.8	0.1	6.3	0.1	8.4	0.3
MLSS (mg/L)	3385		4010		3060	
MLVSS (mg/L)	995		1105		1030	
Run period (day)	1.76		2.00		1.98	
S_0/X_0 (mg NH_4 -N/mg MLVSS)	0.46		0.46		0.45	
$q_{specific}$ (mg NH_4 -N/mg MLVSS.day)	0.23		0.20		0.20	

Table A.35. Operational results of R3 – November 2013.

Run	**1**		**2**		**3**		**4**	
Date	**4.11.2013**	**6.11.2013**	**11.11.2013**	**13.11.2013**	**18.11.2013**	**20.11.2013**	**25.11.2013**	**26.11.2013**
pH	7.73	6.54	7.51	6.5	7.54	6.55	7.27	6.32
Temperature (^{o}C)	22.4	24.3	21.9	25.4	17.7	24.2	20.6	25.1
NH_4-N (mg/L)	470	36	455	60.5	427.5	59.5	507.5	67.5
NH_4 -N removal (%)	92.3		86.7		86.1		86.7	
NH_3 (mg/L)	12.0	0.1	6.8	0.1	5.0	0.1	4.0	0.1
MLSS (mg/L)	3610		3960		3895		4030	
MLVSS (mg/L)	1045		1190		1190		1310	
Run period (day)	1.91		1.94		1.94		1.01	
S_0/X_0 (mg NH_4 -N/mg MLVSS)	0.45		0.38		0.36		0.39	
$q_{specific}$ (mg NH_4 -N/mg MLVSS.day)	0.22		0.17		0.16		0.33	

Table A.36. Operational results of R3 – December 2013.

Run	**1**		**2**		**3**		**4**	
Date	**2.12.2013**	**3.12.2013**	**9.12.2013**	**10.12.2013**	**16.12.2013**	**17.12.2013**	**23.12.2013**	**25.12.2013**
pH	7.73	6.72	7.55	6.68	7.45	6.7	7.61	6.54
Temperature (^{o}C)	22.3	29.4	18.6	30.6	18.5	30.9	19.5	27.8
NH_4-N (mg/L)	467.5	37	457.5	48	452.5	61.5	475	56.5
NH_4 -N removal (%)	92.1		89.5		86.4		88.1	
NH_3 (mg/L)	11.8	0.2	5.9	0.2	4.6	0.3	7.5	0.1
MLSS (mg/L)	3765		4455		4260		5665	
MLVSS (mg/L)	1195		1235		1245		1370	
Run period (day)	1.00		0.92		0.97		1.93	
S_0/X_0 (mg NH_4 -N/mg MLVSS)	0.39		0.37		0.36		0.35	
$q_{specific}$ (mg NH_4 -N/mg MLVSS.day)	0.36		0.36		0.32		0.16	

Table A.37. Operational results of R3 – January 2014.

Run	**1**		**2**		**3**		**4**	
Date	**6.1.2014**	**7.1.2014**	**13.1.2014**	**14.1.2014**	**20.1.2014**	**21.1.2014**	**27.1.2013**	**28.1.2014**
pH	7.37	6.59	7.46	6.62	7.76	6.76	7.51	6.54
Temperature (^{o}C)	19.3	31	18	31.6	21.8	31	21	31
NH_4-N (mg/L)	470	36	455	60.5	427.5	59.5	507.5	67.5
NH_4 -N removal (%)	92.3		86.7		86.1		86.7	
NH_3 (mg/L)	4.2	0.1	4.6	0.2	11.2	0.3	7.1	0.2
MLSS (mg/L)	5580		4310		4825		5250	
MLVSS (mg/L)	1525		865		1310		1385	
Run period (day)	1.03		0.98		0.94		0.95	
S_0/X_0 (mg NH_4 -N/mg MLVSS)	0.31		0.53		0.33		0.37	
$q_{specific}$ (mg NH_4 -N/mg MLVSS.day)	0.28		0.47		0.30		0.33	

Table A.38. Operational results of R3 – February 2014.

Run	**1**		**2**		**3**		**4**	
Date	**3.2.2014**	**4.2.2014**	**10.2.2014**	**11.2.2014**	**19.2.2014**	**20.2.2014**	**24.2.2014**	**25.2.2014**
pH	7.37	6.68	7.69	7.08	7.55	6.37	7.73	9.19
Temperature (^{o}C)	19.1	31.4	22.6	31.8	19.3	31.2	22.2	31.8
NH_4-N (mg/L)	250	39.5	245	46.5	247.5	19	255	157.5
NH_4 -N removal (%)	84.2		81.0		92.3		38.2	
NH_3 (mg/L)	2.2	0.2	5.8	0.5	3.4	0.0	6.4	93.5
MLSS (mg/L)	3970		3350		4645		4895	
MLVSS (mg/L)	1100		920		1240		1055	
Run period (day)	0.95		0.94		0.96		0.74	
S_0/X_0 (mg NH_4 -N/mg MLVSS)	0.23		0.27		0.20		0.28	
$q_{specific}$ (mg NH_4 -N/mg MLVSS.day)	0.20		0.23		0.19		0.14	

Table A.39. Operational results of R3 – March 2014.

Run	**1**		**2**		**3**	
Date	**3.3.2014**	**4.3.2014**	**10.3.2014**	**11.3.2014**	**26.3.2014**	**27.3.2014**
pH	6.9	7.03	7.05	7.67	7.41	7.57
Temperature (°C)	19.7	30.6	19.5	30	31.6	29.4
NH_4-N (mg/L)	44.25	37	31	28	16	12
NH_4 -N removal (%)	16.4		9.7		25.0	
NH_3 (mg/L)	0.1	0.3	0.1	1.1	0.4	0.3
MLSS (mg/L)	4115		2110		1230	
MLVSS (mg/L)	950		585		405	
Run period (day)	0.91		0.93		1.10	
S_0/X_0 (mg NH_4 -N/mg MLVSS)	0.05		0.05		0.04	
$q_{specific}$ (mg NH_4 -N/mg MLVSS.day)	0.01		0.01		0.01	

Table A.40. Operational results of R3 – April 2014.

Run	**1**		**2**		**3**	
Date	**7.4.2014**	**8.4.2014**	**14.4.2014**	**15.4.2014**	**29.4.2014**	**30.4.2014**
pH	7.8	8.77	7.84	8.14	7.65	8.13
Temperature (°C)	23.9	30.5	19.8	30.3	21.3	22.7
NH_4-N (mg/L)	62.5	35	56	21.5	91	45
NH_4 -N removal (%)	44.0		61.6		50.5	
NH_3 (mg/L)	2.1	11.8	1.5	2.3	1.8	2.8
MLSS (mg/L)	510		485		400	
MLVSS (mg/L)	305		275		315	
Run period (day)	0.97		0.78		0.73	
S_0/X_0 (mg NH_4 -N/mg MLVSS)	0.20		0.20		0.29	
$q_{specific}$ (mg NH_4 -N/mg MLVSS.day)	0.09		0.16		0.20	

Table A.41. Operational results of R3 – May 2014.

Run	**1**		**2**		**3**		**4**	
Date	**6.5.2014**	**7.5.2014**	**12.5.2014**	**13.5.2014**	**20.5.2014**	**21.5.2014**	**27.5.2014**	**28.5.2014**
pH	7.57	6.94	7.75	6.6	7.53	6.31	7.41	6.34
Temperature (^{o}C)	23.1	22.2	20.3	22.1	21.9	22.6	23.3	25.5
NH_4-N (mg/L)	61.5	1	96.5	2.75	127.5	13.75	140	10.25
NH_4 -N removal (%)	98.4		97.2		89.2		92.7	
NH_3 (mg/L)	1.1	0.0	2.2	0.0	2.0	0.0	1.8	0.0
MLSS (mg/L)	425		710		745		785	
MLVSS (mg/L)	275		400		440		480	
Run period (day)	0.75		0.98		0.83		1.17	
S_0/X_0 (mg NH_4 -N/mg MLVSS)	0.22		0.24		0.29		0.35	
$q_{specific}$ (mg NH_4 -N/mg MLVSS.day)	0.29		0.24		0.31		0.28	

Table A.42. Operational results of R3 – June 2014.

Run	**1**		**2**		**3**		**4**	
Date	**3.6.2014**	**4.6.2014**	**11.6.2014**	**12.6.2014**	**17.6.2014**	**18.6.2014**	**23.6.2014**	**24.6.2014**
pH	7.52	6.65	7.48	6.55	7.41	5.75	7.48	6.61
Temperature (^{o}C)	22.4	21.9	23.6	23.1	26.4	25.4	26.5	27.1
NH_4-N (mg/L)	182.5	14.75	202.5	9.75	207.5	11	180	8.75
NH_4 -N removal (%)	91.9		95.2		94.7		95.1	
NH_3 (mg/L)	2.9	0.0	3.2	0.0	3.4	0.0	3.5	0.0
MLSS (mg/L)	1265		1390		1880		1610	
MLVSS (mg/L)	615		745		905		775	
Run period (day)	0.97		0.78		0.97		0.78	
S_0/X_0 (mg NH_4 -N/mg MLVSS)	0.30		0.27		0.23		0.23	
$q_{specific}$ (mg NH_4 -N/mg MLVSS.day)	0.28		0.33		0.22		0.28	

Table A.43. Operational results of R3 – July 2014.

Run	**1**		**2**		**3**	
Date	**2.7.2014**	**3.7.2014**	**9.7.2014**	**11.7.2014**	**16.7.2014**	**17.7.2014**
pH	7.5	5.99	7.89	6.38	7.7	5.8
Temperature (^{o}C)	23.6	24.2	25.1	26.1	25.5	26.1
NH_4-N (mg/L)	207.5	10.5	257.5	9	217.5	9.25
NH_4 -N removal (%)	94.9		96.5		95.7	
NH_3 (mg/L)	3.4	0.0	11.3	0.0	6.4	0.0
MLSS (mg/L)	1835		1715		2325	
MLVSS (mg/L)	800		905		1075	
Run period (day)	0.95		1.93		0.95	
S_0/X_0 (mg NH_4 -N/mg MLVSS)	0.26		0.28		0.20	
$q_{specific}$ (mg NH_4 -N/mg MLVSS.day)	0.26		0.14		0.20	

Table A.44. Operational results of R3 – August 2014.

Run	**1**		**2**		**3**		**4**	
Date	**6.8.2014**	**7.8.2014**	**13.8.2014**	**14.8.2014**	**18.8.2014**	**19.8.2014**	**25.8.2014**	**26.8.2014**
pH	7.74	6.47	7.72	6.24	7.53	6.14	7.4	6.44
Temperature (^{o}C)	27.5	27.4	27	27	26.2	26.3	25.9	26
NH_4-N (mg/L)	185	2.25	250	4.75	235	15	235	12
NH_4 -N removal (%)	98.8		98.1		93.6		94.9	
NH_3 (mg/L)	6.9	0.0	8.6	0.0	5.0	0.0	3.6	0.0
MLSS (mg/L)	2755		3580		3165		3020	
MLVSS (mg/L)	1295		1560		1295		1225	
Run period (day)	0.95		0.93		0.95		0.95	
S_0/X_0 (mg NH_4 -N/mg MLVSS)	0.14		0.16		0.18		0.19	
$q_{specific}$ (mg NH_4 -N/mg MLVSS.day)	0.15		0.17		0.18		0.19	

Table A.45. Operational results of R3 – September 2014.

Run	**1**		**2**	
Date	**1.9.2014**	**2.9.2014**	**8.9.2014**	**9.9.2014**
pH	7.93	6.75	7.55	6.38
Temperature (^{o}C)	25.2	25.3	27.4	25
NH_4-N (mg/L)	242.5	16.5	222.5	8.5
NH_4 -N removal (%)	93.2		96.2	
NH_3 (mg/L)	11.7	0.1	5.4	0.0
MLSS (mg/L)	3125		3940	
MLVSS (mg/L)	960		1560	
Run period (day)	0.93		0.92	
S_0/X_0 (mg NH_4 -N/mg MLVSS)	0.25		0.14	
$q_{specific}$ (mg NH_4 -N/mg MLVSS.day)	0.25		0.15	

Phase 2 – OPERATION OF REACTORS WITH DIFFERENT SUBSTRATES

Control Reactor (CR): a mixture consisting of glucose, peptone and sodium acetate,

Glucose Reactor (RG): glucose only, Peptone Reactor (RP): peptone only

These reactors had the COD/TKN ratio of 10

Table A.46. Operational results of CR – July 2013.

Run	**1**		**2**		**3**		**4**		**5**	
Date	**1.7.2013**	**3.7.2013**	**8.7.2013**	**10.7.2013**	**15.7.2013**	**17.7.2013**	**22.7.2013**	**24.7.2013**	**29.7.2013**	**31.7.2013**
pH	7.92	8.64	8.07	8.64	8.15	8.47	7.82	8.51	8.37	8.67
Temperature (^{o}C)	24.3	24.2	24.2	24.6	24	23.9	23.2	25	25.4	27.6
COD (mg/L)	772	120	850	91	911	80	884	87	933	86
COD removal (%)	84.5		89.3		91.2		90.2		90.8	
MLSS (mg/L)	4320		4665		4450		4515		3785	
MLVSS (mg/L)	3235		3465		3405		3360		2830	
Run period (day)	1.92		1.94		1.75		1.93		1.76	
F:M ratio (mg COD/mg MLVSS.day)	0.12		0.13		0.15		0.14		0.19	
S_0/X_0 (mg COD/mg MLVSS)	0.24		0.25		0.27		0.26		0.33	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.10		0.11		0.14		0.12		0.17	

Table A.47. Operational results of CR – August 2013.

Run	**1**		**2**		**3**		**4**	
Date	**5.8.2013**	**7.8.2013**	**12.8.2013**	**14.8.2013**	**19.8.2013**	**21.8.2013**	**26.8.2013**	**28.8.2013**
pH	8.03	8.7	8.28	8.33	7.85	8.75	8.17	8.96
Temperature (^{o}C)	25	26.2	26.2	26.5	25.9	26.5	25.6	26.3
COD (mg/L)	881	79	974	75	988	95	964	97
COD removal (%)	91.0		92.3		90.4		89.9	
MLSS (mg/L)	4400		4475		4475		4065	
MLVSS (mg/L)	3145		3405		3270		2775	
Run period (day)	1.94		1.79		1.95		1.93	
F:M ratio (mg COD/mg MLVSS.day)	0.14		0.16		0.15		0.18	
S_0/X_0 (mg COD/mg MLVSS)	0.28		0.29		0.30		0.35	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.13		0.15		0.14		0.16	

Table A.48. Operational results of CR – September 2013.

Run	**1**		**2**		**3**		**4**		**5**	
Date	**2.9.2013**	**4.9.2013**	**9.9.2013**	**11.9.2013**	**16.9.2013**	**18.9.2013**	**23.9.2013**	**25.9.2013**	**30.9.2013**	**2.10.2013**
pH	8.15	8.75	8.15	9.25	8.76	9.3	8.36	8.95	7.79	8.23
Temperature (^{o}C)	23.5	24	21.8	23.1	23.2	24.7	20.7	22.1	21.5	20
COD (mg/L)	1039	113	1045	91	1086	151	1093	175	1061	161
COD removal (%)	89.1		91.3		86.1		84.0		84.8	
MLSS (mg/L)	3705		3110		3435		3760		4275	
MLVSS (mg/L)	2750		2315		2515		2830		3175	
Run period (day)	1.94		1.86		1.96		1.94		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.19		0.24		0.22		0.20		0.17	
S_0/X_0 (mg COD/mg MLVSS)	0.38		0.45		0.43		0.39		0.33	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.17		0.22		0.19		0.17		0.14	

Table A.49.Operational results of CR – October 2013.

Run	**1**		**2**		**3**	
Date	**7.10.2013**	**9.10.2013**	**21.10.2013**	**23.10.2013**	**28.10.2013**	**30.10.2013**
pH	6.78	7.81	7.41	7.47	7.48	7.57
Temperature (^{o}C)	20.2	23.7	22.5	24.9	22.9	24.8
COD (mg/L)	1010	122	1084	151	1068	92
COD removal (%)	87.9		86.1		91.4	
MLSS (mg/L)	3830		4645		4045	
MLVSS (mg/L)	3000		3485		2915	
Run period (day)	1.77		2.01		1.99	
F:M ratio (mg COD/mg MLVSS.day)	0.19		0.15		0.18	
S_0/X_0 (mg COD/mg MLVSS)	0.34		0.31		0.37	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.17		0.13		0.17	

Table A.50. Operational results of CR – November 2013.

Run	**1**		**2**		**3**		**4**	
Date	**4.11.2013**	**6.11.2013**	**11.11.2013**	**13.11.2013**	**18.11.2013**	**20.11.2013**	**25.11.2013**	**27.11.2013**
pH	6.83	7.06	6.54	6.71	6.26	6.54	6.57	6.14
Temperature (^{o}C)	22.3	25.3	23.5	23.6	20.5	23.3	22.8	23.2
COD (mg/L)	1009	74	1024	95	1002	89	988	148
COD removal (%)	92.7		90.7		91.1		85.0	
MLSS (mg/L)	3885		3670		3920		4145	
MLVSS (mg/L)	2930		2775		3005		3115	
Run period (day)	1.92		1.95		1.95		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.18		0.19		0.17		0.16	
S_0/X_0 (mg COD/mg MLVSS)	0.34		0.37		0.33		0.32	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.17		0.17		0.16		0.14	

Table A.51. Operational results of CR – December 2013.

Run	**1**		**2**		**3**		**4**	
Date	**2.12.2013**	**4.12.2013**	**9.12.2013**	**11.12.2013**	**16.12.2013**	**18.12.2013**	**23.12.2013**	**25.12.2013**
pH	6.31	6.25	5.99	5.74	5.83	5.63	5.73	5.45
Temperature (^{o}C)	23.7	22.8	20.4	22.6	22.7	23.1	23.1	24.8
COD (mg/L)	1012	177	1015	88	946	79	993	67
COD removal (%)	82.5		91.3		91.6		93.3	
MLSS (mg/L)	4015		3015		3605		4270	
MLVSS (mg/L)	3010		2205		2680		3185	
Run period (day)	1.95		1.97		1.96		1.94	
F:M ratio (mg COD/mg MLVSS.day)	0.17		0.23		0.18		0.16	
S_0/X_0 (mg COD/mg MLVSS)	0.34		0.46		0.35		0.31	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.14		0.21		0.17		0.15	

Table A.52. Operational results of CR – January 2014.

Run	**1**		**2**		**3**		**4**	
Date	**6.1.2014**	**8.1.2014**	**13.1.2014**	**15.1.2014**	**20.1.2014**	**22.1.2014**	**27.1.2014**	**29.1.2014**
pH	5.68	4.99	6.25	5.16	5.66	4.94	6.26	4.88
Temperature (^{o}C)	22	25.4	21.6	25.6	23.4	24.4	22.4	25
COD (mg/L)	941	59	994	46	878	58	1020	55
COD removal (%)	93.7		95.4		93.4		94.6	
MLSS (mg/L)	3670		3785		4610		4135	
MLVSS (mg/L)	2295		2580		3585		3170	
Run period (day)	1.97		1.92		1.96		1.95	
F:M ratio (mg COD/mg MLVSS.day)	0.21		0.20		0.12		0.17	
S_0/X_0 (mg COD/mg MLVSS)	0.41		0.39		0.24		0.32	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.20		0.19		0.12		0.16	

Table A.53. Operational results of CR – February 2014.

Run	**1**		**2**		**3**		**4**	
Date	**3.2.2014**	**5.2.2014**	**10.2.2014**	**12.2.2014**	**17.2.2014**	**19.2.2014**	**24.2.2014**	**26.2.2014**
pH	5.66	4.77	6.37	4.91	6.11	5.47	6.28	5.8
Temperature (^{o}C)	21.8	24	23.1	25.8	21	25.5	23.3	24
COD (mg/L)	1023	70	1031	102	1021	138	986	192
COD removal (%)	93.2		90.1		86.5		80.5	
MLSS (mg/L)	3655		3445		3455		3460	
MLVSS (mg/L)	2900		2830		2765		2760	
Run period (day)	1.95		1.95		1.94		1.78	
F:M ratio (mg COD/mg MLVSS.day)	0.18		0.19		0.19		0.20	
S_0/X_0 (mg COD/mg MLVSS)	0.35		0.36		0.37		0.36	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.17		0.17		0.16		0.16	

Table A.54. Operational results of CR – March 2014.

Run	**1**		**2**		**3**		**4**	
Date	**3.3.2014**	**5.3.2014**	**10.3.2014**	**12.3.2014**	**24.3.2014**	**26.3.2014**	**31.3.2014**	**2.4.2014**
pH	6.14	5.87	6.24	6.28	7.42	7.71	7.93	7.75
Temperature (^{o}C)	22.4	24.2	21.8	23.7	22.8	24.1	22.3	22.9
COD (mg/L)	1013	191	907	283	923	91	956	108
COD removal (%)	81.1		68.8		90.1		88.7	
MLSS (mg/L)	3165		2625		2915		4365	
MLVSS (mg/L)	2390		1980		2045		3055	
Run period (day)	1.78		1.78		1.80		1.80	
F:M ratio (mg COD/mg MLVSS.day)	0.24		0.26		0.25		0.17	
S_0/X_0 (mg COD/mg MLVSS)	0.42		0.46		0.45		0.31	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.19		0.18		0.23		0.15	

Table A.55. Operational results of CR – April 2014.

Run	**1**		**2**		**3**	
Date	**7.4.2014**	**9.4.2014**	**14.4.2014**	**16.4.2014**	**28.4.2014**	**30.4.2014**
pH	7.83	8.01	8	8.19	7.89	8.51
Temperature (^{o}C)	22.4	22.1	23	24.3	19.8	22.1
COD (mg/L)	944	102	986	97	909	105
COD removal (%)	89.2		90.2		88.4	
MLSS (mg/L)	5085		5015		5070	
MLVSS (mg/L)	3440		3435		3800	
Run period (day)	1.80		1.78		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.15		0.16		0.12	
S_0/X_0 (mg COD/mg MLVSS)	0.27		0.29		0.24	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.14		0.15		0.11	
	9.4.2014	**11.4.2014**	**28.4.2014**	**29.4.2014**	**30.4.2014**	
Surface charge (meqv/g MLSS)	-0.055	-0.061	-0.058	-0.062	-0.062	
Hydrophobicity (%)	49	50	41	40	37	

Table A.56. Operational results of CR – May 2014.

Run	**1**		**2**		**3**	
Date	**5.5.2014**	**7.5.2014**	**12.5.2014**	**14.5.2014**	**26.5.2014**	**28.5.2014**
pH	7.61	8.1	7.66	8.13	8.02	8.62
Temperature (°C)	22.1	22	20.9	21.8	22.4	26.1
COD (mg/L)	899	67	922	107	965	69
COD removal (%)	92.5		88.4		92.8	
MLSS (mg/L)	4500		4765		4930	
MLVSS (mg/L)	3205		3575		3045	
Run period (day)	1.78		1.80		2.15	
F:M ratio (mg COD/mg MLVSS.day)	0.16		0.14		0.15	
S_0/X_0 (mg COD/mg MLVSS)	0.28		0.26		0.32	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.15		0.13		0.14	
	21.5.2014					
Surface charge (meqv/g MLSS)	-0.067					
Hydrophobicity (%)	40					

Table A.57. Operational results of CR – June 2014.

Run	**1**		**2**		**3**		**4**	
Date	**2.6.2014**	**4.6.2014**	**10.6.2014**	**12.6.2014**	**16.6.2014**	**18.6.2014**	**23.6.2014**	**25.6.2014**
pH	7.78	8.18	7.9	8.42	7.7	8.43	7.71	8.46
Temperature (°C)	21.6	21.6	21.8	23.3	23.8	25.2	22.3	24.1
COD (mg/L)	937	80	974	138	998	103	601	76
COD removal (%)	91.5		85.8		89.7		87.4	
MLSS (mg/L)	4975		5270		5180		4330	
MLVSS (mg/L)	3745		3870		3900		3080	
Run period (day)	1.97		1.96		1.96		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.13		0.13		0.13		0.10	
S_0/X_0 (mg COD/mg MLVSS)	0.25		0.25		0.26		0.20	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.12		0.11		0.12		0.09	
	6.6.2014				**24.6.2014**			
Surface charge (meqv/g MLSS)	-0.063				-0.085			
Hydrophobicity (%)	53				43			

Table A.58. Operational results of CR – July 2014.

Run	**1**		**2**		**3**	
Date	**30.6.2014**	**2.7.2014**	**7.7.2014**	**9.7.2014**	**14.7.2014**	**16.7.2014**
pH	7.64	7.98	7.23	8.22	7.6	8.11
Temperature (°C)	20.5	24.1	23.6	25.5	25.3	25.9
COD (mg/L)	831	70	935	71	825	71
COD removal (%)	91.6		92.4		91.4	
MLSS (mg/L)	4545		4300		4605	
MLVSS (mg/L)	2800		3190		3170	
Run period (day)	1.94		1.97		1.97	
F:M ratio (mg COD/mg MLVSS.day)	0.15		0.15		0.13	
S_0/X_0 (mg COD/mg MLVSS)	0.30		0.29		0.26	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.14		0.14		0.12	
	3.7.2014	**8.7.2014**	**10.7.2014**		**15.7.2014**	
Surface charge (meqv/g MLSS)	-0.144	-0.108	-0.137		-0.098	
Hydrophobicity (%)	48	58	64		60	

Table A.59. Operational results of CR – August 2014.

Run	**1**		**2**		**3**		**4**	
Date	**4.8.2014**	**6.8.2014**	**11.8.2014**	**13.8.2014**	**18.8.2014**	**20.8.2014**	**25.8.2014**	**27.8.2014**
pH	7.15	8.05	7.59	8.2	7.3	8.11	7.79	8.25
Temperature (°C)	26.5	27.3	22.4	26.9	25.1	25.7	24.1	26
COD (mg/L)	825	47	960	41	951	47	981	28
COD removal (%)	94.3		95.7		95.1		97.1	
MLSS (mg/L)	4560		4870		5020		5180	
MLVSS (mg/L)	3095		3650		3460		3605	
Run period (day)	1.95		1.94		1.97		2.00	
F:M ratio (mg COD/mg MLVSS.day)	0.14		0.14		0.14		0.14	
S_0/X_0 (mg COD/mg MLVSS)	0.27		0.26		0.27		0.27	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.13		0.13		0.13		0.13	
	5.8.2014	**7.8.2014**	**12.8.2014**		**19.8.2014**	**21.8.2014**	**26.8.2014**	**28.8.2014**
Surface charge (meqv/g MLSS)	-0.073	-0.094	-0.108		-0.087	-0.095	-0.09	-0.076
Hydrophobicity (%)	53	74	71		54	60	50	66

Table A.60. Operational results of CR – September 2014.

Run	**1**		**2**	
Date	**1.9.2014**	**3.9.2014**	**8.9.2014**	**10.9.2014**
pH	7.42	8.15	7.83	8.05
Temperature (°C)	23.7	25.3	24.1	24.7
COD (mg/L)	960	223	891	69
COD removal (%)	76.8		92.3	
MLSS (mg/L)	4745		4345	
MLVSS (mg/L)	3235		2960	
Run period (day)	1.96		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.15		0.15	
S_0/X_0 (mg COD/mg MLVSS)	0.30		0.30	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.12		0.14	
	2.9.2014		**4.9.2014**	
Surface charge (meqv/g MLSS)	-0.075		-0.068	
Hydrophobicity (%)	54		58	

Table A.61. Operational results of RG – July 2013.

Run	**1**		**2**		**3**		**4**		**5**	
Date	**1.7.2013**	**3.7.2013**	**8.7.2013**	**10.7.2013**	**15.7.2013**	**17.7.2013**	**22.7.2013**	**24.7.2013**	**29.7.2013**	**31.7.2013**
pH	6.86	6.25	6.39	6.6	7.31	7.07	8.41	8.53	7.84	8.72
Temperature (°C)	21.8	25.1	23.5	23.5	23.4	22.8	21.8	23.5	24.2	26.4
COD (mg/L)	905	50	934	87	612	61	989	51	833	49
COD removal (%)	94.5		90.7		90.0		94.8		94.1	
NH_4-N (mg/L)	121.5	80	119.5	75.5	58.5	7	61	0	63.5	0
NH_4 -N removal (%)	34.2		36.8		88.0		100.0		100.0	
MLSS (mg/L)	3845		3220		3430		3225		3825	
MLVSS (mg/L)	3240		2655		2805		2690		3010	
Run period (day)	1.93		1.95		1.76		1.93		1.76	
F:M ratio (mg COD/mg MLVSS.day)	0.14		0.18		0.12		0.19		0.16	
S_0/X_0 (mg COD/mg MLVSS)	0.28		0.35		0.22		0.37		0.28	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.14		0.16		0.11		0.18		0.15	

Table A.62. Operational results of RG – August 2013.

Run	**1**		**2**		**3**		**4**	
Date	**5.8.2013**	**7.8.2013**	**12.8.2013**	**14.8.2013**	**19.8.2013**	**21.8.2013**	**26.8.2013**	**28.8.2013**
pH	7.31	8.84	6.82	7.97	7.24	8.84	7.64	8.53
Temperature (^{o}C)	24	25	25.6	25.1	25.5	25.7	24.9	25.7
COD (mg/L)	776	45	806	19	783	43	761	27
COD removal (%)	94.2		97.6		94.5		96.5	
NH_4-N (mg/L)	63.5	0	66	0	72.5	0		
NH_4 -N removal (%)	100.0		100.0		100.0			
MLSS (mg/L)	4195		4020		4345		4490	
MLVSS (mg/L)	3240		3255		3500		3455	
Run period (day)	1.95		1.80		1.95		1.94	
F:M ratio (mg COD/mg MLVSS.day)	0.12		0.14		0.11		0.11	
S_0/X_0 (mg COD/mg MLVSS)	0.24		0.25		0.22		0.22	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.12		0.13		0.11		0.11	

Table A.63. Operational results of RG – September 2013.

Run	**1**		**2**		**3**		**4**		**5**	
Date	**2.9.2013**	**4.9.2013**	**9.9.2013**	**11.9.2013**	**16.9.2013**	**18.9.2013**	**23.9.2013**	**25.9.2013**	**30.9.2013**	**2.10.2013**
pH	7.77	8.71	6.72	5.59	5.55	4.76	6.58	6.89	7.01	7.29
Temperature (^{o}C)	23.1	22.5	21.6	23	22.1	23.9	18.8	21.4	20.5	19.5
COD (mg/L)	837	28	838	13	906	80	819	54	870	39
COD removal (%)	96.7		98.4		91.2		93.4		95.5	
MLSS (mg/L)	4615		3805		4155		3360		3995	
MLVSS (mg/L)	3620		3360		3665		2870		3235	
Run period (day)	1.94		1.87		1.97		1.95		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.12		0.13		0.13		0.15		0.14	
S_0/X_0 (mg COD/mg MLVSS)	0.23		0.25		0.25		0.29		0.27	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.12		0.13		0.11		0.14		0.13	

Table A.64. Operational results of RG – October 2013.

Run	**1**		**2**		**3**	
Date	**7.10.2013**	**9.10.2013**	**21.10.2013**	**23.10.2013**	**28.10.2013**	**30.10.2013**
pH	6.81	7.01	6.7	6.4	7.07	6.16
Temperature (°C)	19.4	21.1	18.8	18.8	19.5	19.7
COD (mg/L)	770	47	830	310	858	66
COD removal (%)	93.9		62.7		92.3	
MLSS (mg/L)	3180		3175		3515	
MLVSS (mg/L)	2680		2625		2645	
Run period (day)	1.79		2.01		1.99	
F:M ratio (mg COD/mg MLVSS.day)	0.16		0.16		0.16	
S_0/X_0 (mg COD/mg MLVSS)	0.29		0.32		0.32	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.15		0.10		0.15	

Table A.65. Operational results of RG – November 2013.

Run	**1**		**2**		**3**		**4**	
Date	**4.11.2013**	**6.11.2013**	**11.11.2013**	**13.11.2013**	**18.11.2013**	**20.11.2013**	**25.11.2013**	**27.11.2013**
pH	6.8	5.97	6.57	5.69	6.29	5.82	6.71	6.67
Temperature (°C)	20.8	25.7	21.3	22.9	18.4	21.5	21.1	22.7
COD (mg/L)	803	21	942	41	906	41	1434	163
COD removal (%)	97.4		95.6		95.5		88.6	
MLSS (mg/L)	2635		2540		2065		1745	
MLVSS (mg/L)	2285		2080		1745		1435	
Run period (day)	1.92		1.96		1.96		1.97	
F:M ratio (mg COD/mg MLVSS.day)	0.18		0.23		0.26		0.51	
S_0/X_0 (mg COD/mg MLVSS)	0.35		0.45		0.52		1.00	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.18		0.22		0.25		0.45	

Table A.66. Operational results of RG – December 2013.

Run	**1**		**2**		**3**		**4**	
Date	**2.12.2013**	**4.12.2013**	**9.12.2013**	**11.12.2013**	**16.12.2013**	**18.12.2013**	**23.12.2013**	**25.12.2013**
pH	6.93	5.83	6.61	5.51	6.15	5.57	6.28	5.45
Temperature (^{o}C)	22.1	21.1	18.1	22.3	21.3	22.7	21	24.8
COD (mg/L)	1382	94	1298	22	1167	33	730	40
COD removal (%)	93.2		98.3		97.2		94.5	
MLSS (mg/L)	3405		3755		4415		4585	
MLVSS (mg/L)	2865		3130		3540		3620	
Run period (day)	1.95		1.98		1.96		1.94	
F:M ratio (mg COD/mg MLVSS.day)	0.25		0.21		0.17		0.10	
S_0/X_0 (mg COD/mg MLVSS)	0.48		0.41		0.33		0.20	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.23		0.21		0.16		0.10	

Table A.67. Operational results of RG – January 2014.

Run	**1**		**2**		**3**		**4**	
Date	**6.1.2014**	**8.1.2014**	**13.1.2014**	**15.1.2014**	**20.1.2014**	**22.1.2014**	**27.1.2014**	**29.1.2014**
pH	5.74	5.23	6.09	5.41	6.83	5.25	6.52	5.18
Temperature (^{o}C)	21.2	23.4	18.2	20.7	21.9	20.1	20.3	23.7
COD (mg/L)	790	24	723	15	732	25	795	19
COD removal (%)	97.0		97.9		96.6		97.6	
MLSS (mg/L)	5235		5090		5530		4775	
MLVSS (mg/L)	4250		3875		4635		3970	
Run period (day)	1.98		1.93		1.96		1.95	
F:M ratio (mg COD/mg MLVSS.day)	0.09		0.10		0.08		0.09	
S_0/X_0 (mg COD/mg MLVSS)	0.19		0.19		0.16		0.17	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.09		0.09		0.08		0.08	

Table A.68. Operational results of RG – February 2014.

Run	**1**		**2**		**3**		**4**	
Date	**3.2.2014**	**5.2.2014**	**10.2.2014**	**12.2.2014**	**17.2.2014**	**19.2.2014**	**24.2.2014**	**26.2.2014**
pH	5.89	5.46	6.87	5.52	6.94	5.34	6.21	5.57
Temperature (^{o}C)	21.6	19.5	22.7	26.2	18.5	26.1	21	25.2
COD (mg/L)	771	15	847	23	878	32	956	54
COD removal (%)	98.1		97.3		96.4		94.4	
MLSS (mg/L)	4205		3475		3850		3410	
MLVSS (mg/L)	3550		2955		3300		2825	
Run period (day)	1.95		1.96		1.95		1.79	
F:M ratio (mg COD/mg MLVSS.day)	0.11		0.15		0.14		0.16	
S_0/X_0 (mg COD/mg MLVSS)	0.22		0.29		0.27		0.28	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.11		0.14		0.13		0.15	

Table A.69. Operational results of RG – March 2014.

Run	**1**		**2**		**3**		**4**	
Date	**3.3.2014**	**5.3.2014**	**10.3.2014**	**12.3.2014**	**24.3.2014**	**26.3.2014**	**31.3.2014**	**2.4.2014**
pH	5.83	5.43	6.97	5.37	6.52	5.94	7.53	5.71
Temperature (^{o}C)	22.4	26.3	19.6	20.6	20.1	22.6	20.3	24.7
COD (mg/L)	753	6	976	37	918	45	972	64
COD removal (%)	99.2		96.2		95.1		93.4	
MLSS (mg/L)	3275		3790		3515		3935	
MLVSS (mg/L)	2620		3085		2855		3190	
Run period (day)	1.78		1.79		1.80		1.80	
F:M ratio (mg COD/mg MLVSS.day)	0.16		0.18		0.18		0.14	
S_0/X_0 (mg COD/mg MLVSS)	0.29		0.32		0.32		0.25	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.16		0.17		0.17		0.13	
	19.3.2014		**21.3.2014**		**27.3.2014**			
Surface charge (meqv/g MLSS)	-0.081		-0.069		-0.115			
Hydrophobicity (%)	62		63		53			

Table A.70. Operational results of RG – April 2014.

Run	**1**		**2**		**3**	
Date	**7.4.2014**	**9.4.2014**	**14.4.2014**	**16.4.2014**	**28.4.2014**	**30.4.2014**
pH	6.85	5.71	6.87	5.69	6.8	5.51
Temperature (°C)	22.2	24.3	19.8	25.5	18.9	21.8
COD (mg/L)	748	53	918	13	905	85
COD removal (%)	92.9		98.6		90.6	
MLSS (mg/L)	3645		4505		4040	
MLVSS (mg/L)	2945		3630		3495	
Run period (day)	1.80		1.79		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.14		0.14		0.13	
S_0/X_0 (mg COD/mg MLVSS)	0.25		0.25		0.26	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.13		0.14		0.12	

Table A.71. Operational results of RG – May 2014.

Run	**1**		**2**		**3**	
Date	**5.5.2014**	**7.5.2014**	**12.5.2014**	**14.5.2014**	**26.5.2014**	**28.5.2014**
pH	6.43	5.78	5.92	5.94	7.47	5.66
Temperature (°C)	20.6	21.1	19.9	22.2	19.3	23.8
COD (mg/L)	837	88	661	79	876	29
COD removal (%)	89.5		88.0		96.7	
MLSS (mg/L)	3785		3740		3850	
MLVSS (mg/L)	3085		3140		3455	
Run period (day)	1.78		1.81		2.15	
F:M ratio (mg COD/mg MLVSS.day)	0.15		0.12		0.12	
S_0/X_0 (mg COD/mg MLVSS)	0.27		0.21		0.25	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.14		0.10		0.11	
	7.5.2014		**9.5.2014**		**14.5.2014**	**16.5.2014**
Surface charge (meqv/g MLSS)	-0.081		-0.084		-0.088	-0.08
Hydrophobicity (%)	58		67		71	66

Table A.72. Operational results of RG – June 2014.

Run	**1**		**2**		**3**		**4**	
Date	**2.6.2014**	**4.6.2014**	**10.6.2014**	**12.6.2014**	**16.6.2014**	**18.6.2014**	**23.6.2014**	**25.6.2014**
pH	6.61	5.64	6.94	5.82	6.36	5.86	5.97	6.02
Temperature (^{o}C)	21.7	20.9	20.3	23	24.3	24.7	22.3	24.1
COD (mg/L)	809	15	981	25	851	52	909	89
COD removal (%)	98.1		97.5		93.9		90.2	
MLSS (mg/L)	4055		4635		4520		3895	
MLVSS (mg/L)	3540		4030		3995		3325	
Run period (day)	1.97		1.97		1.96		1.97	
F:M ratio (mg COD/mg MLVSS.day)	0.12		0.12		0.11		0.14	
S_0/X_0 (mg COD/mg MLVSS)	0.23		0.24		0.21		0.27	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.11		0.12		0.10		0.13	
	6.6.2014				**24.6.2014**			
Surface charge (meqv/g MLSS)	-0.058				-0.115			
Hydrophobicity (%)	29				45			

Table A.73. Operational results of RG – July 2014.

Run	**1**		**2**		**3**	
Date	**30.6.2014**	**2.7.2014**	**7.7.2014**	**9.7.2014**	**14.7.2014**	**16.7.2014**
pH	6.27	6.07	6.29	5.85	6.09	5.77
Temperature (^{o}C)	27.1	23.9	22.3	24.5	25.4	25
COD (mg/L)	705	14	904	48	847	55
COD removal (%)	98.0		94.7		93.5	
MLSS (mg/L)	3370		3810		3325	
MLVSS (mg/L)	2890		3300		2800	
Run period (day)	1.95		1.97		1.97	
F:M ratio (mg COD/mg MLVSS.day)	0.13		0.14		0.15	
S_0/X_0 (mg COD/mg MLVSS)	0.24		0.27		0.30	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.12		0.13		0.14	
	3.7.2014		**8.7.2014**		**10.7.2014**	**15.7.2014**
Surface charge (meqv/g MLSS)	-0.108		-0.132		-0.103	-0.13
Hydrophobicity (%)	55		65		74	71

Table A.74. Operational results of RG – August 2014.

Run	**1**		**2**		**3**		**4**	
Date	**4.8.2014**	**6.8.2014**	**11.8.2014**	**13.8.2014**	**18.8.2014**	**20.8.2014**	**25.8.2014**	**27.8.2014**
pH	6.31	5.84	5.96	5.62	6.79	5.79	6.47	5.78
Temperature (^{o}C)	25.9	26.2	21.6	25.3	23.4	26.3	23.9	25.9
COD (mg/L)	835	44	798	54	923	52	822	33
COD removal (%)	94.7		93.2		94.4		96.0	
MLSS (mg/L)	3645		4065		4510		4940	
MLVSS (mg/L)	3125		3485		3855		4210	
Run period (day)	1.95		1.94		1.98		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.14		0.12		0.12		0.10	
S_0/X_0 (mg COD/mg MLVSS)	0.27		0.23		0.24		0.20	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.13		0.11		0.11		0.10	
	5.8.2014	**7.8.2014**	**12.8.2014**		**19.8.2014**	**21.8.2014**	**26.8.2014**	**28.8.2014**
Surface charge (meqv/g MLSS)	-0.1	-0.098	-0.083		-0.091	-0.081	-0.099	-0.092
Hydrophobicity (%)	51	38	55		53	56	54	50

Table A.75. Operational results of RG – September 2014.

Run	**1**		**2**	
Date	**1.9.2014**	**3.9.2014**	**8.9.2014**	**10.9.2014**
pH	6.17	5.64	6.82	5.64
Temperature (^{o}C)	24.2	23.9	22.5	24.1
COD (mg/L)	794	56	921	67
COD removal (%)	92.9		92.7	
MLSS (mg/L)	4560		3790	
MLVSS (mg/L)	3805		3240	
Run period (day)	1.96		1.97	
F:M ratio (mg COD/mg MLVSS.day)	0.11		0.14	
S_0/X_0 (mg COD/mg MLVSS)	0.21		0.28	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.10		0.13	
	2.9.2014		**4.9.2014**	
Surface charge (meqv/g MLSS)	-0.089		-0.087	
Hydrophobicity (%)	42		56	

Table A.76. Operational results of RP – July 2013.

Run	**1**		**2**		**3**		**4**		**5**	
Date	**1.7.2013**	**3.7.2013**	**8.7.2013**	**10.7.2013**	**15.7.2013**	**17.7.2013**	**22.7.2013**	**24.7.2013**	**29.7.2013**	**31.7.2013**
pH	7.96	7.86	8.02	8.3	7.95	7.91	7.92	8.19	8.08	8.28
Temperature (^{o}C)	23.3	23.8	23.8	24.3	23.6	24.2	22.5	25	24.8	27.8
COD (mg/L)	804	120	719	124	547	75	570	95	709	52
COD removal (%)	85.1		82.8		86.3		83.3		92.7	
MLSS (mg/L)	3725		4015		3350		2985		3065	
MLVSS (mg/L)	2830		2950		2455		2110		1995	
Run period (day)	1.91		1.93		1.75		1.92		1.75	
F:M ratio (mg COD/mg MLVSS.day)	0.15		0.13		0.13		0.14		0.20	
S_0/X_0 (mg COD/mg MLVSS)	0.28		0.24		0.22		0.27		0.36	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.13		0.10		0.11		0.12		0.19	

Table A.77. Operational results of RP – August 2013.

Run	**1**		**2**		**3**		**4**	
Date	**5.8.2013**	**7.8.2013**	**12.8.2013**	**14.8.2013**	**19.8.2013**	**21.8.2013**	**26.8.2013**	**28.8.2013**
pH	7.97	8.35	7.88	8.19	7.96	8.44	7.98	8.18
Temperature (^{o}C)	24.4	26.1	25.3	26.6	25.2	26	24.8	26.3
COD (mg/L)	599	95	611	25	541	48	614	53
COD removal (%)	84.1		95.9		91.1		91.4	
MLSS (mg/L)	3295		3220		3740		3415	
MLVSS (mg/L)	2040		2115		2440		2175	
Run period (day)	1.93		1.79		1.94		1.93	
F:M ratio (mg COD/mg MLVSS.day)	0.15		0.16		0.11		0.15	
S_0/X_0 (mg COD/mg MLVSS)	0.29		0.29		0.22		0.28	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.13		0.15		0.10		0.13	

Table A.78. Operational results of RP – September 2013.

Run	**1**		**2**		**3**		**4**		**5**	
Date	**2.9.2013**	**4.9.2013**	**9.9.2013**	**11.9.2013**	**16.9.2013**	**18.9.2013**	**23.9.2013**	**25.9.2013**	**30.9.2013**	**2.10.2013**
pH	8.02	8.05	8.12	8.44	8.2	8.62	8.21	8.55	8.03	8.19
Temperature (^{o}C)	23.4	22.9	20.5	22.9	22.3	23.8	18.9	21.7	20.3	19.8
COD (mg/L)	465	57	882	33	934	74	875	88	761	72
COD removal (%)	87.7		96.3		92.1		89.9		90.5	
MLSS (mg/L)	3435		3775		5280		5165		5050	
MLVSS (mg/L)	2240		2535		3315		3075		3095	
Run period (day)	1.93		1.86		1.96		1.94		1.95	
F:M ratio (mg COD/mg MLVSS.day)	0.11		0.19		0.14		0.15		0.13	
S_0/X_0 (mg COD/mg MLVSS)	0.21		0.35		0.28		0.28		0.25	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.09		0.18		0.13		0.13		0.11	

Table A.79. Operational results of RP – October 2013.

Run	**1**		**2**		**3**	
Date	**7.10.2013**	**9.10.2013**	**21.10.2013**	**23.10.2013**	**28.10.2013**	**30.10.2013**
pH	7.8	7.87	8.07	8.14	7.89	8.4
Temperature (^{o}C)	21.4	23.3	22	24.4	21.5	21.4
COD (mg/L)	773	57	1016	83	886	56
COD removal (%)	92.6		91.8		93.7	
MLSS (mg/L)	5345		6020		5105	
MLVSS (mg/L)	3260		3570		3060	
Run period (day)	1.77		2.01		1.98	
F:M ratio (mg COD/mg MLVSS.day)	0.13		0.14		0.15	
S_0/X_0 (mg COD/mg MLVSS)	0.24		0.28		0.29	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.12		0.13		0.14	

Table A.80. Operational results of RP – November 2013.

Run	**1**		**2**		**3**		**4**	
Date	**4.11.2013**	**6.11.2013**	**11.11.2013**	**13.11.2013**	**18.11.2013**	**20.11.2013**	**25.11.2013**	**27.11.2013**
pH	7.71	8.08	7.84	8.39	8.05	8.64	7.54	7.73
Temperature (^{o}C)	20.2	25	21.6	24.7	18.2	20.4	19.6	22.1
COD (mg/L)	978	45	726	66	938	68	1354	153
COD removal (%)	95.4		90.9		92.8		88.7	
MLSS (mg/L)	4905		4100		4910		2410	
MLVSS (mg/L)	3080		2555		2950		1430	
Run period (day)	1.91		1.95		1.95		1.95	
F:M ratio (mg COD/mg MLVSS.day)	0.17		0.15		0.16		0.49	
S_0/X_0 (mg COD/mg MLVSS)	0.32		0.28		0.32		0.95	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.16		0.13		0.15		0.43	

Table A.81. Operational results of RP – December 2013.

Run	**1**		**2**		**3**		**4**	
Date	**2.12.2013**	**4.12.2013**	**9.12.2013**	**11.12.2013**	**16.12.2013**	**18.12.2013**	**23.12.2013**	**25.12.2013**
pH	8.11	8.53	7.73	8.01	7.67	7.71	7.7	7.71
Temperature (^{o}C)	20.3	22.8	17.7	20.6	19.9	22.2	20.2	21.4
COD (mg/L)	1504	203	1329	59	1175	89	851	76
COD removal (%)	86.5		95.6		92.4		91.1	
MLSS (mg/L)	3540		3865		4185		5145	
MLVSS (mg/L)	2195		2275		2580		3180	
Run period (day)	1.94		1.97		1.95		1.93	
F:M ratio (mg COD/mg MLVSS.day)	0.35		0.30		0.23		0.14	
S_0/X_0 (mg COD/mg MLVSS)	0.69		0.58		0.46		0.27	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.31		0.28		0.22		0.13	

Table A.82. Operational results of RP – January 2014.

Run	**1**		**2**		**3**		**4**	
Date	**6.1.2014**	**8.1.2014**	**13.1.2014**	**15.1.2014**	**20.1.2014**	**22.1.2014**	**27.1.2014**	**29.1.2014**
pH	7.52	7.47	7.74	7.87	7.9	7.87	7.5	7.14
Temperature (°C)	19.4	22.3	18.7	23.6	21.6	22	19.3	22.3
COD (mg/L)	888	66	822	53	984	65	1016	75
COD removal (%)	92.6		93.6		93.4		92.6	
MLSS (mg/L)	5080		5940		6740		6690	
MLVSS (mg/L)	3305		3490		4385		4335	
Run period (day)	1.97		1.92		1.95		1.95	
F:M ratio (mg COD/mg MLVSS.day)	0.14		0.12		0.12		0.12	
S_0/X_0 (mg COD/mg MLVSS)	0.27		0.24		0.22		0.23	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.13		0.11		0.11		0.11	

Table A.83. Operational results of RP – February 2014.

Run	**1**		**2**		**3**		**4**	
Date	**3.2.2014**	**5.2.2014**	**10.2.2014**	**12.2.2014**	**17.2.2014**	**19.2.2014**	**24.2.2014**	**26.2.2014**
pH	7.38	7.37	7.53	7.2	7.22	7.61	6.92	6.81
Temperature (°C)	20.3	24.4	22.3	23.4	18.2	23.2	22.8	22.3
COD (mg/L)	928	186	1038	145	1031	46	1018	128
COD removal (%)	80.0		86.0		95.5		87.4	
MLSS (mg/L)	5520		5735		6205		5240	
MLVSS (mg/L)	3640		4010		4255		4145	
Run period (day)	1.95		1.95		1.94		1.78	
F:M ratio (mg COD/mg MLVSS.day)	0.13		0.13		0.12		0.14	
S_0/X_0 (mg COD/mg MLVSS)	0.25		0.26		0.24		0.25	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.10		0.11		0.12		0.12	

Table A.84. Operational results of RP – March 2014.

Run	**1**		**2**		**3**		**4**	
Date	**3.3.2014**	**5.3.2014**	**10.3.2014**	**12.3.2014**	**24.3.2014**	**26.3.2014**	**31.3.2014**	**2.4.2014**
pH	7.68	7.89	7.77	8.57	7.98	8.25	8.17	8.43
Temperature (^{o}C)	21.5	22.8	21	23.2	21.9	25	19.9	22.6
COD (mg/L)	726	64	852	52	801	54	867	66
COD removal (%)	91.2		93.9		93.3		92.4	
MLSS (mg/L)	5520		5735		6205		5240	
MLVSS (mg/L)	3640		4010		4255		4145	
Run period (day)	1.78		1.79		1.79		1.79	
F:M ratio (mg COD/mg MLVSS.day)	0.11		0.12		0.11		0.12	
S_0/X_0 (mg COD/mg MLVSS)	0.20		0.21		0.19		0.21	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.10		0.11		0.10		0.11	
	19.3.2014				**21.3.2014**			
Surface charge (meqv/g MLSS)	-0.072				-0.087			
Hydrophobicity (%)	56				49			

Table A.85. Operational results of RP – April 2014.

Run	**1**		**2**		**3**	
Date	**7.4.2014**	**9.4.2014**	**14.4.2014**	**16.4.2014**	**28.4.2014**	**30.4.2014**
pH	8.13	8.16	8.25	8.43	8	8.46
Temperature (^{o}C)	20.9	21.1	20.9	22.6	19.4	22.6
COD (mg/L)	676	47	923	42	910	139
COD removal (%)	93.0		95.4		84.7	
MLSS (mg/L)	5265		5900		5625	
MLVSS (mg/L)	3050		3330		3505	
Run period (day)	1.79		1.78		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.12		0.16		0.13	
S_0/X_0 (mg COD/mg MLVSS)	0.22		0.28		0.26	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.12		0.15		0.11	
	2.4.2014		**3.4.2014**		**10.4.2014**	
Surface charge (meqv/g MLSS)	-0.046		-0.063		-0.047	
Hydrophobicity (%)	49		48		52	

Table A.86. Operational results of RP – May 2014.

Run	**1**		**2**		**3**	
Date	**5.5.2014**	**7.5.2014**	**12.5.2014**	**14.5.2014**	**26.5.2014**	**28.5.2014**
pH	8.11	8.39	8.2	8.18	8.07	8.19
Temperature (^{o}C)	22	22.4	20	22.4	20.7	25.5
COD (mg/L)	774	81	769	82	803	67
COD removal (%)	89.5		89.3		91.7	
MLSS (mg/L)	5380		4345		5590	
MLVSS (mg/L)	3190		2800		3655	
Run period (day)	1.78		1.80		2.14	
F:M ratio (mg COD/mg MLVSS.day)	0.14		0.15		0.10	
S_0/X_0 (mg COD/mg MLVSS)	0.24		0.27		0.22	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.12		0.14		0.09	
	9.5.2014			**30.5.2014**		
Surface charge (meqv/g MLSS)	-0.049			-0.064		
Hydrophobicity (%)	75			67		

Table A.87. Operational results of RP – June 2014.

Run	**1**		**2**		**3**		**4**	
Date	**2.6.2014**	**4.6.2014**	**10.6.2014**	**12.6.2014**	**16.6.2014**	**18.6.2014**	**23.6.2014**	**25.6.2014**
pH	8.19	8.12	8.05	8.34	8	8.18	8.01	8.31
Temperature (^{o}C)	20.7	22.1	21.7	23.8	23.6	25.6	22	24.6
COD (mg/L)	816	50	906	93	813	76	852	57
COD removal (%)	93.9		89.7		90.7		93.3	
MLSS (mg/L)	5725		6260		5885		5540	
MLVSS (mg/L)	3670		4130		3840		3405	
Run period (day)	1.97		1.96		1.96		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.11		0.11		0.11		0.13	
S_0/X_0 (mg COD/mg MLVSS)	0.22		0.22		0.21		0.25	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.11		0.10		0.10		0.12	
	3.6.2014	**5.6.2014**	**6.6.2014**	**11.6.2014**	**13.6.2014**	**19.6.2014**	**24.6.2014**	**26.6.2014**
Surface charge (meqv/g MLSS)	-0.051	-0.062	-0.05	-0.056	-0.058	-0.055	-0.049	-0.058
Hydrophobicity (%)	60	65	65	56	54	65	59	59

Table A.88. Operational results of RP – July 2014.

Run	**1**		**2**		**3**	
Date	**30.6.2014**	**2.7.2014**	**7.7.2014**	**9.7.2014**	**14.7.2014**	**16.7.2014**
pH	7.94	8.07	7.98	8.25	7.84	8.13
Temperature (°C)	20.4	24.5	23.7	25.6	25	26.1
COD (mg/L)	816	41	868	76	831	42
COD removal (%)	95.0		91.2		94.9	
MLSS (mg/L)	5365		5780		5635	
MLVSS (mg/L)	3550		3680		3290	
Run period (day)	1.94		1.97		1.97	
F:M ratio (mg COD/mg MLVSS.day)	0.12		0.12		0.13	
S_0/X_0 (mg COD/mg MLVSS)	0.23		0.24		0.25	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.11		0.11		0.12	
	3.7.2014		**8.7.2014**		**10.7.2014**	**15.7.2014**
Surface charge (meqv/g MLSS)	-0.057		-0.064		-0.068	-0.064
Hydrophobicity (%)	69		73		80	57

Table A.89. Operational results of RP – August 2014.

Run	**1**		**2**		**3**		**4**	
Date	**4.8.2014**	**6.8.2014**	**11.8.2014**	**13.8.2014**	**18.8.2014**	**20.8.2014**	**25.8.2014**	**27.8.2014**
pH	7.76	8.13	7.96	8.14	8.11	8.6	8.09	8.36
Temperature (°C)	26.1	28	22.3	27.3	25	26.4	23.5	26.5
COD (mg/L)	842	50	877	50	811	68	923	124
COD removal (%)	94.1		94.3		91.6		86.6	
MLSS (mg/L)	5385		5500		6275		6275	
MLVSS (mg/L)	3430		3605		3720		4020	
Run period (day)	1.95		1.94		1.97		1.95	
F:M ratio (mg COD/mg MLVSS.day)	0.13		0.13		0.11		0.12	
S_0/X_0 (mg COD/mg MLVSS)	0.25		0.24		0.22		0.23	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.12		0.12		0.10		0.10	
	5.8.2014	**7.8.2014**	**12.8.2014**		**19.8.2014**	**21.8.2014**	**26.8.2014**	**28.8.2014**
Surface charge (meqv/g MLSS)	-0.048	-0.059	-0.081		-0.065	-0.062	-0.06	-0.056
Hydrophobicity (%)	71	75	71		69	74	71	75

Table A.90. Operational results of RP – September 2014.

Run	**1**		**2**	
Date	**1.9.2014**	**3.9.2014**	**8.9.2014**	**10.9.2014**
pH	8.08	8.33	8.05	8.28
Temperature (^{o}C)	23.6	25.5	23.7	24.9
COD (mg/L)	846	85	874	143
COD removal (%)	90.0		83.6	
MLSS (mg/L)	5775		5360	
MLVSS (mg/L)	3275		3220	
Run period (day)	1.96		1.96	
F:M ratio (mg COD/mg MLVSS.day)	0.13		0.14	
S_0/X_0 (mg COD/mg MLVSS)	0.26		0.27	
$q_{specific}$ (mg COD/mg MLVSS.day)	0.12		0.12	
	2.9.2014		**4.9.2014**	
Surface charge (meqv/g MLSS)	-0.052		-0.069	
Hydrophobicity (%)	76		68	

APPENDIX B: RAW DATA OF RESPIROMETRIC TESTS

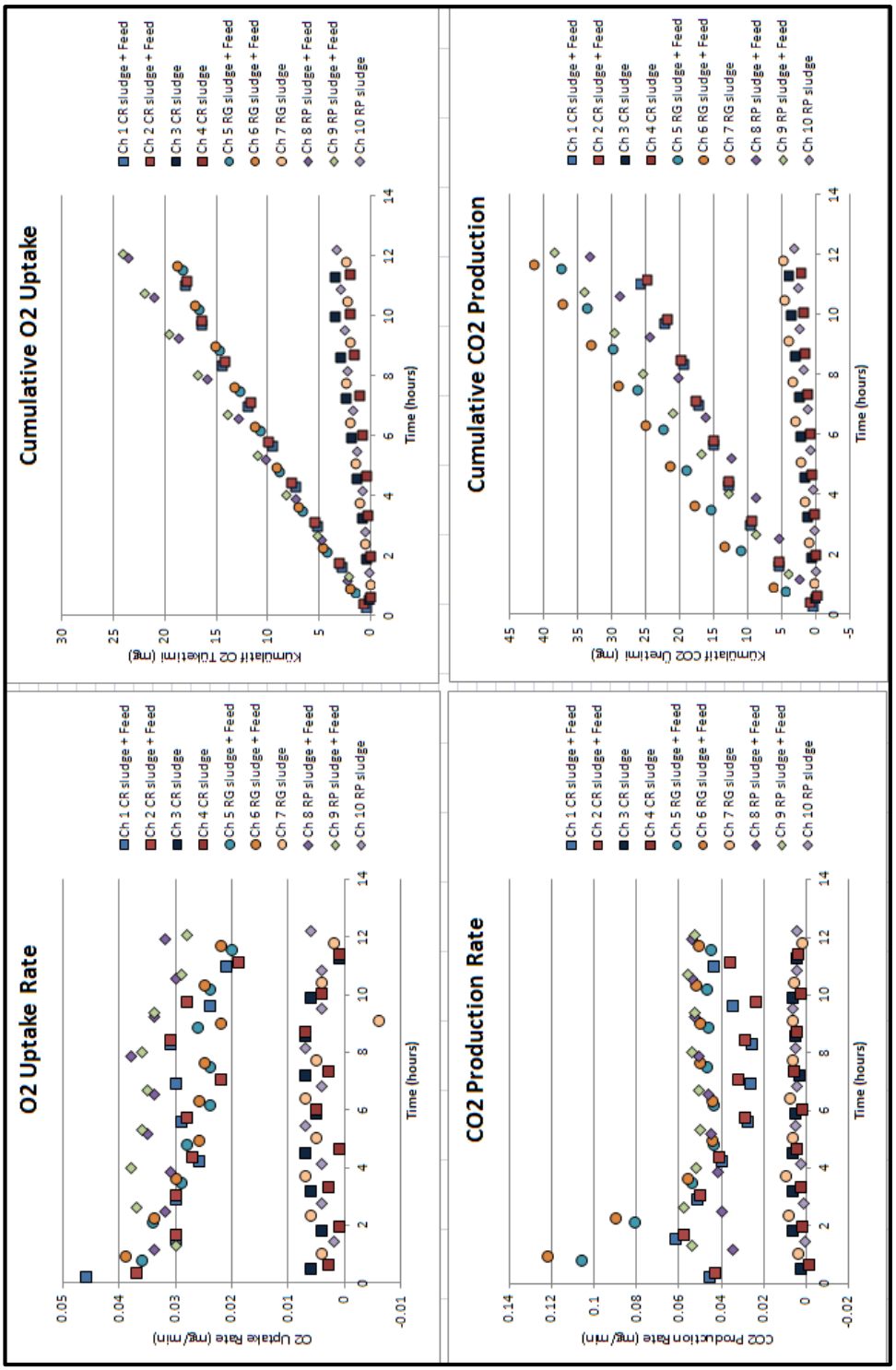

Figure B.1. Raw data of Test 1 (CR, RG, RP – 06.11.2013).

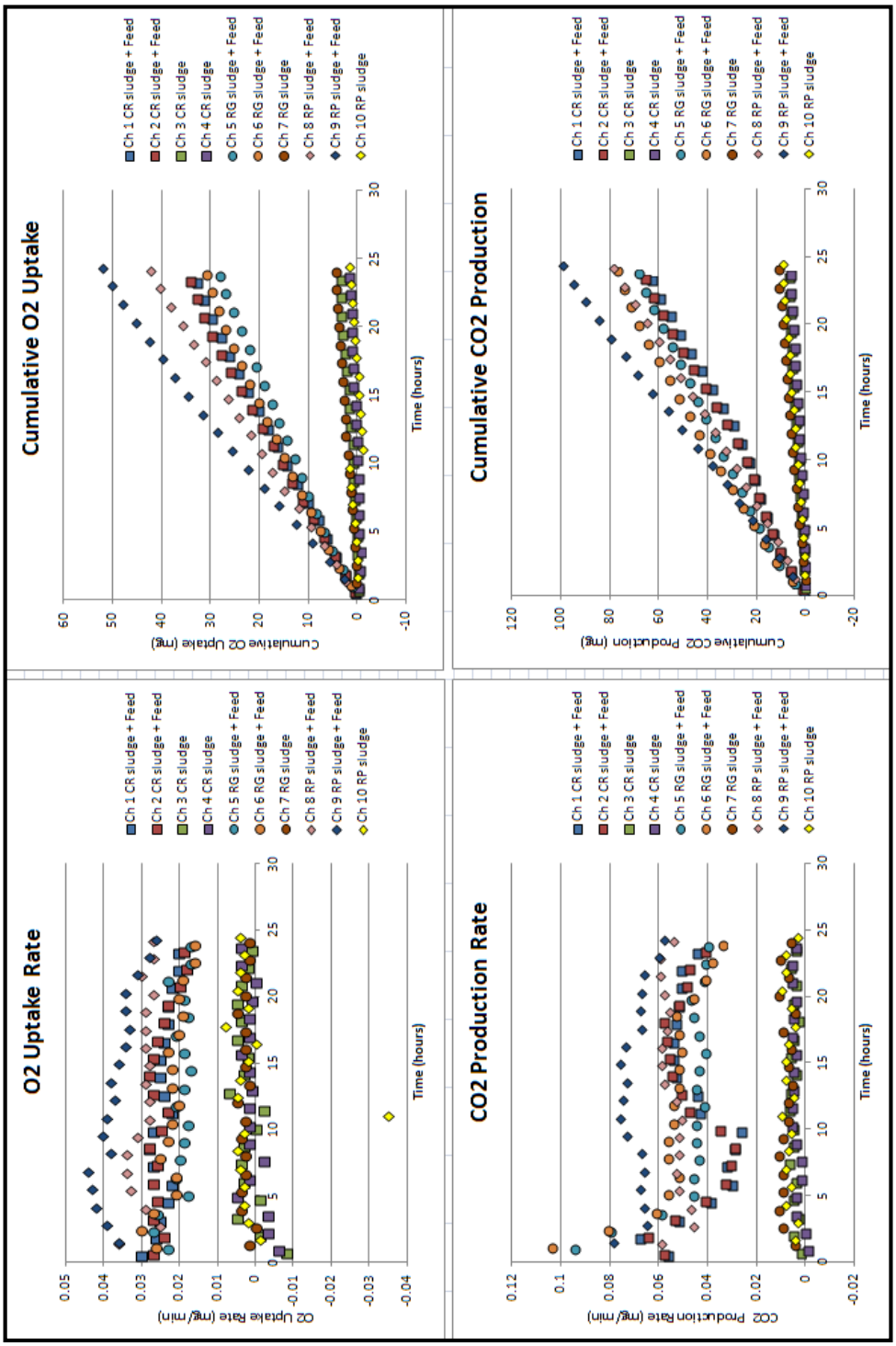

Figure B.2. Raw data of Test 2 (CR, RG, RP – 13.11.2013).

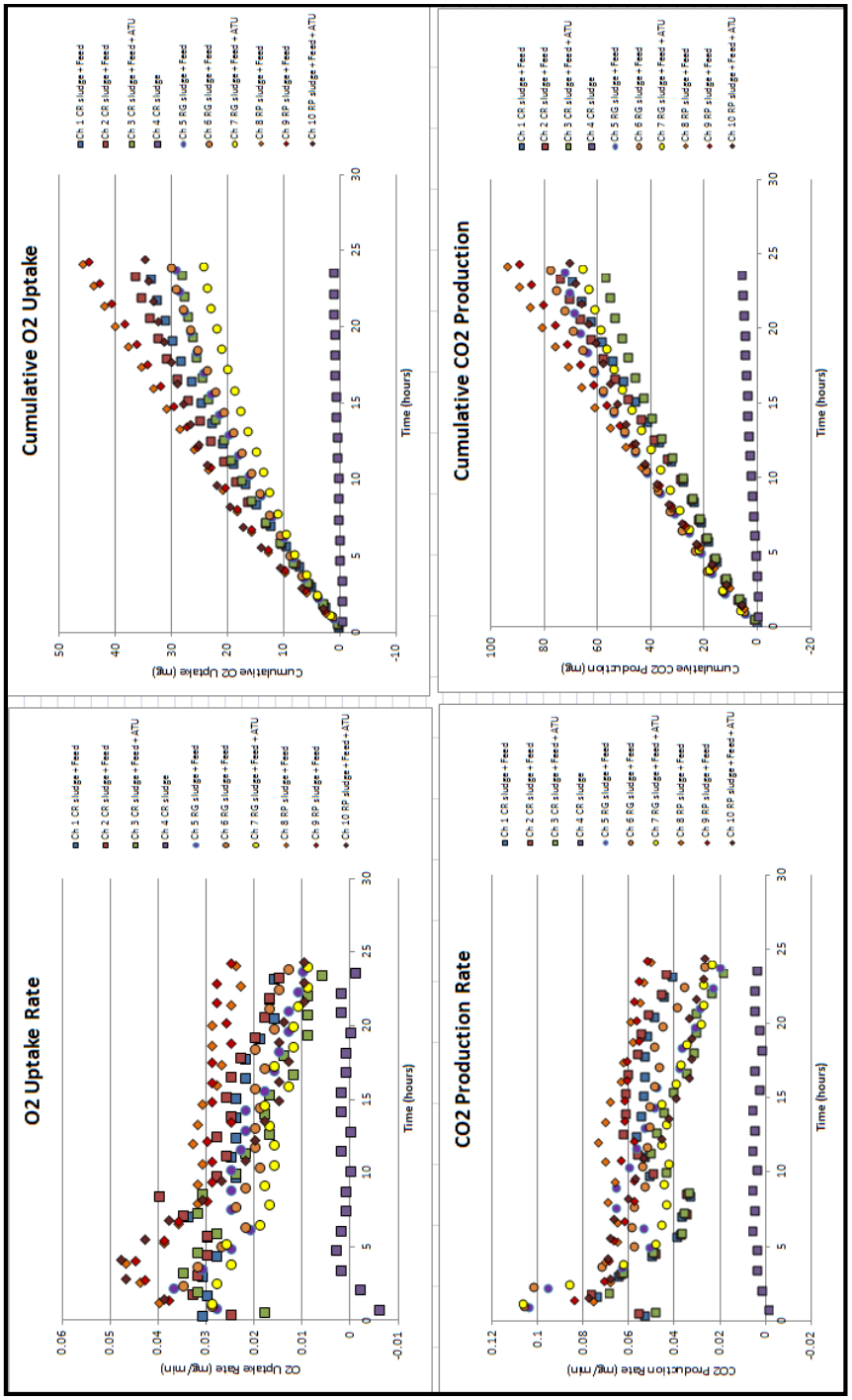

Figure B.3. Raw data of Test 3 (CR, RG, RP – 20.11.2013).

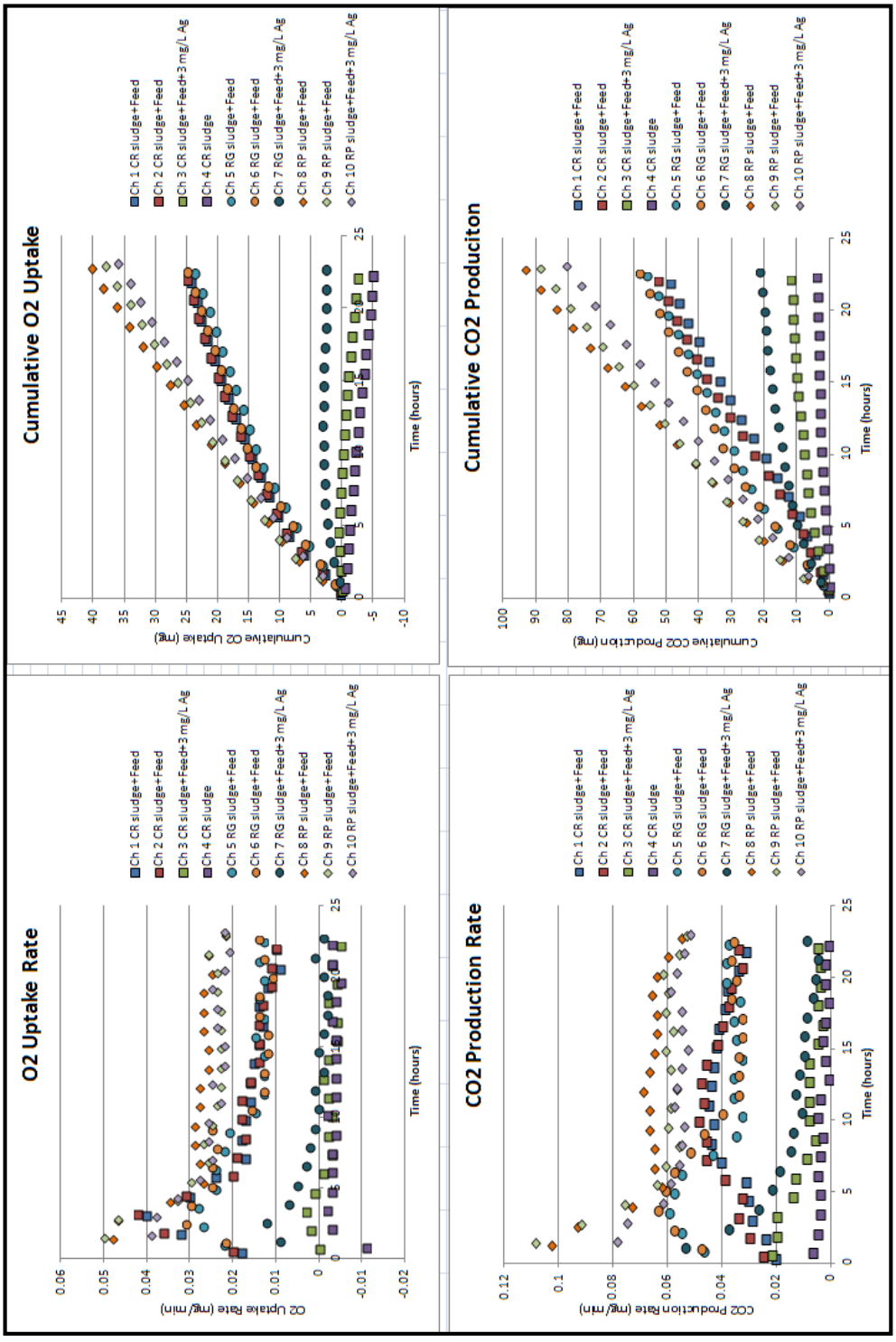

Figure B.4. Raw data of Test 4 (CR, RG, RP – 21.01.2014).

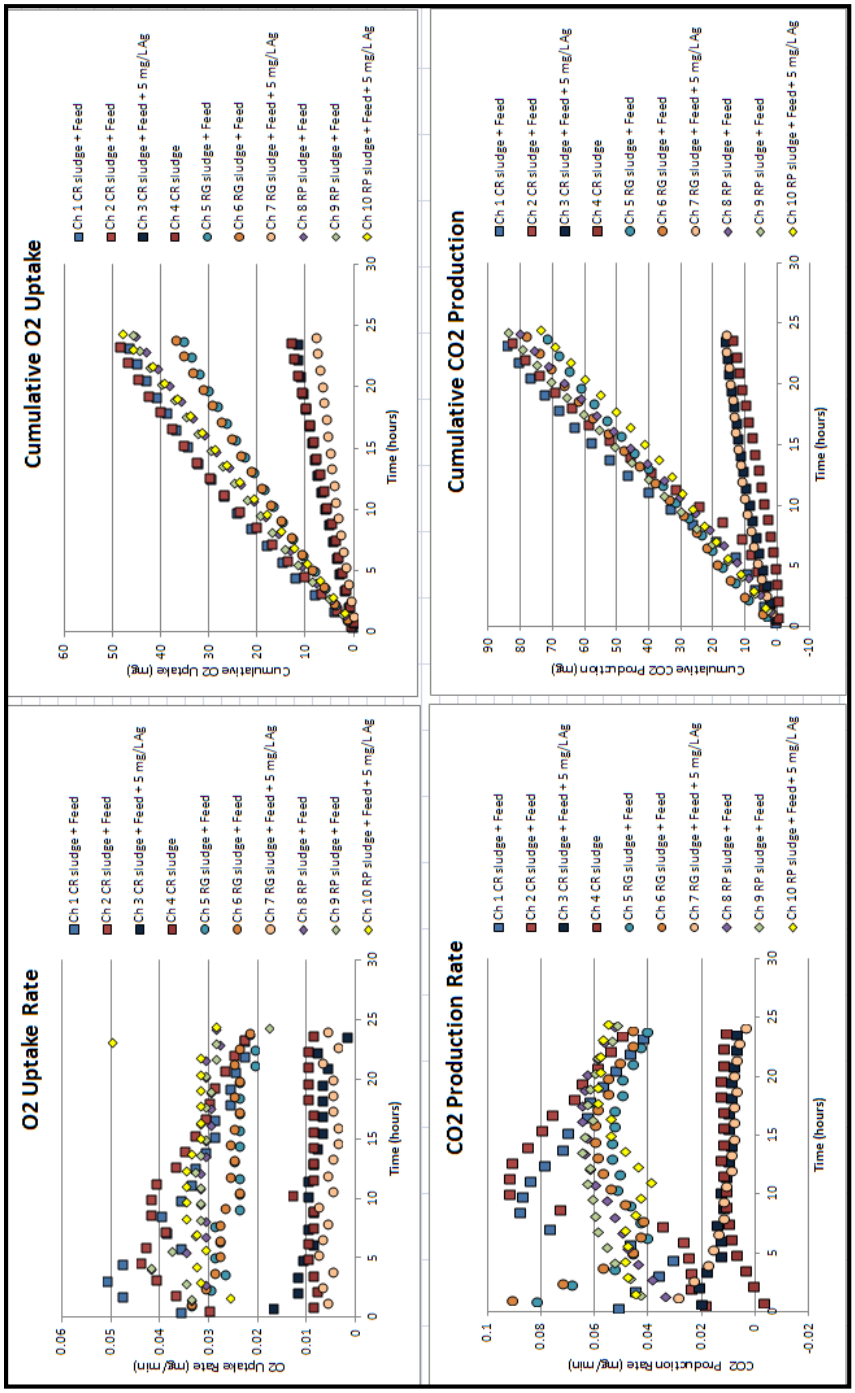

Figure B.5. Raw data of Test 5 (CR, RG, RP – 03.07.2014).

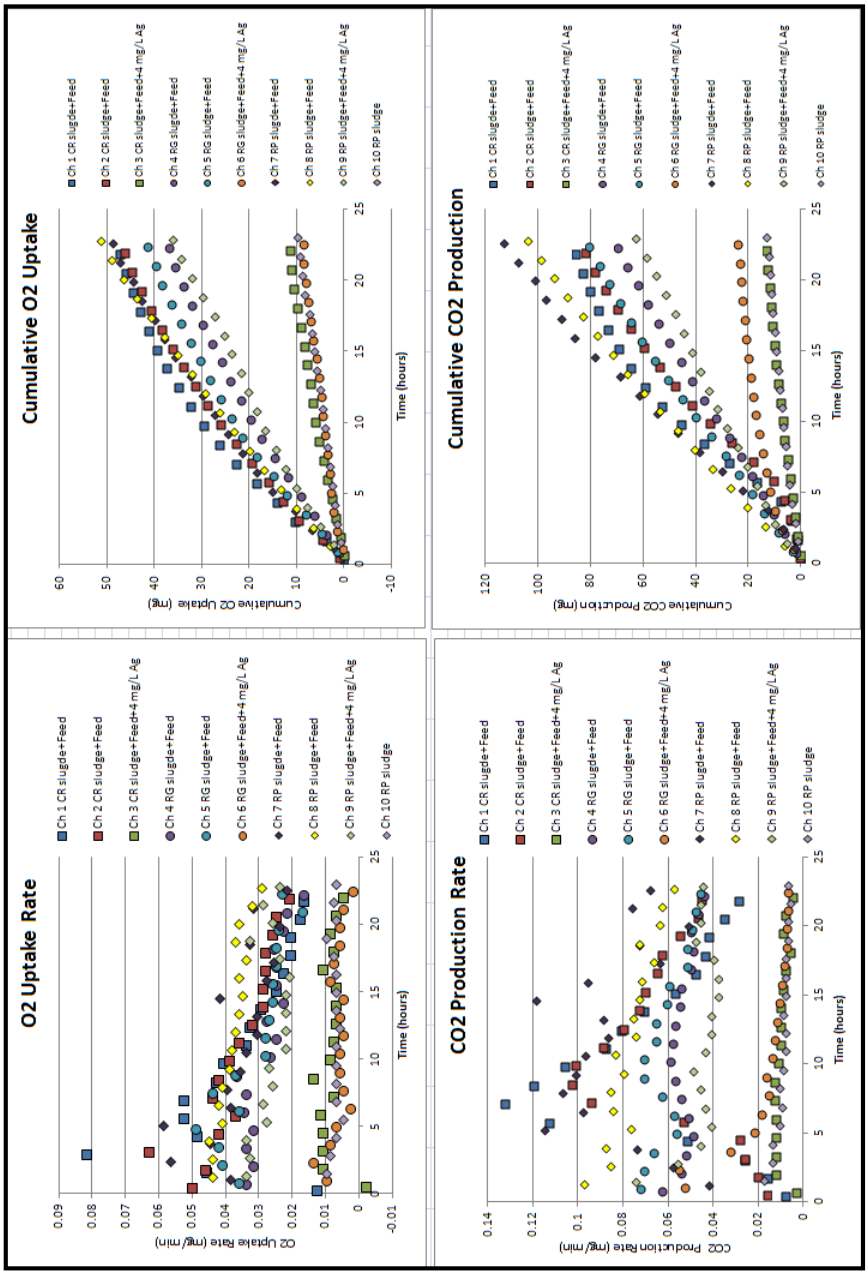

Figure B.6. Raw data of Test 6 (CR, RG, RP – 08.07.2014).

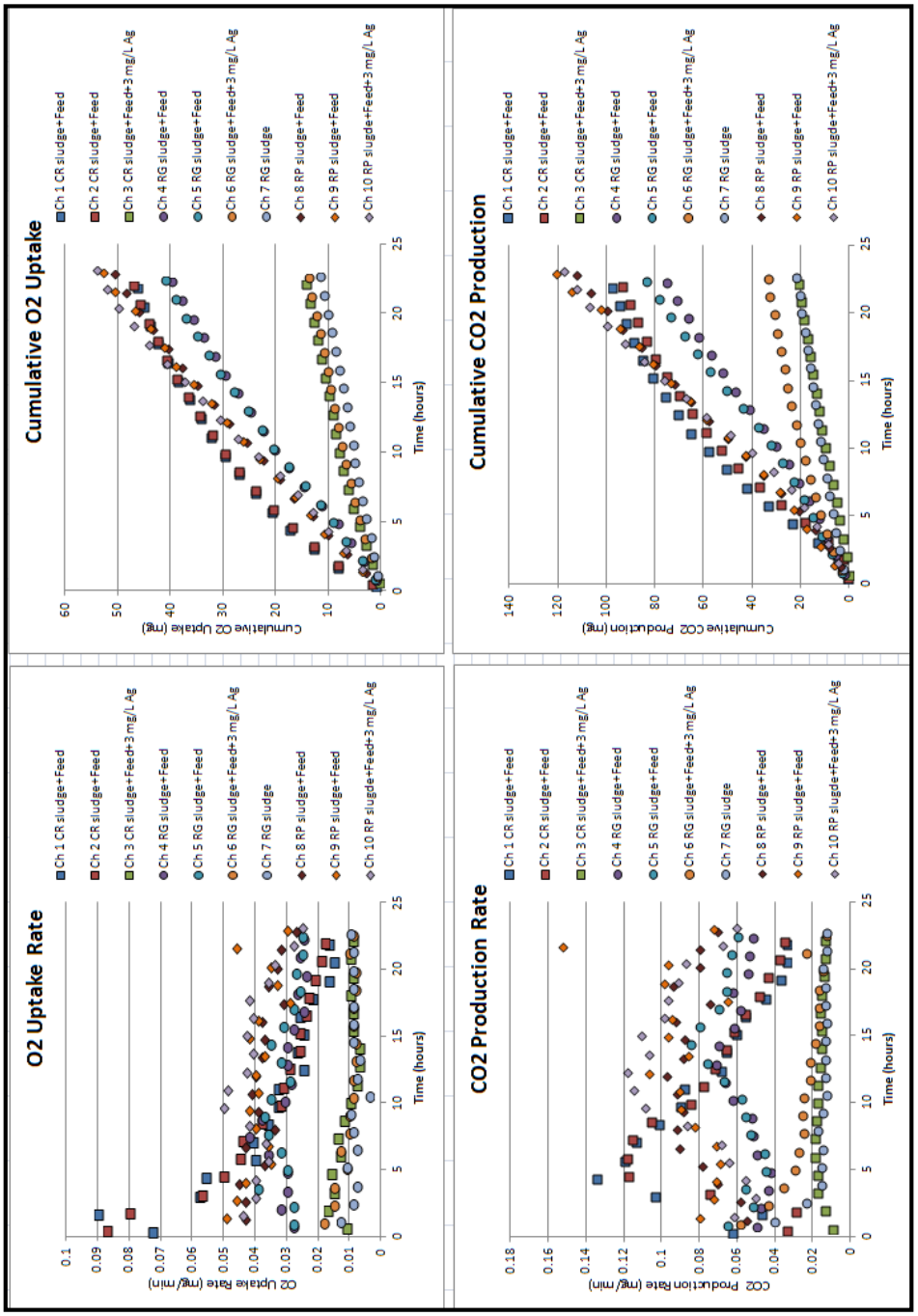

Figure B.7. Raw data of Test 7 (CR, RG, RP – 10.07.2014).

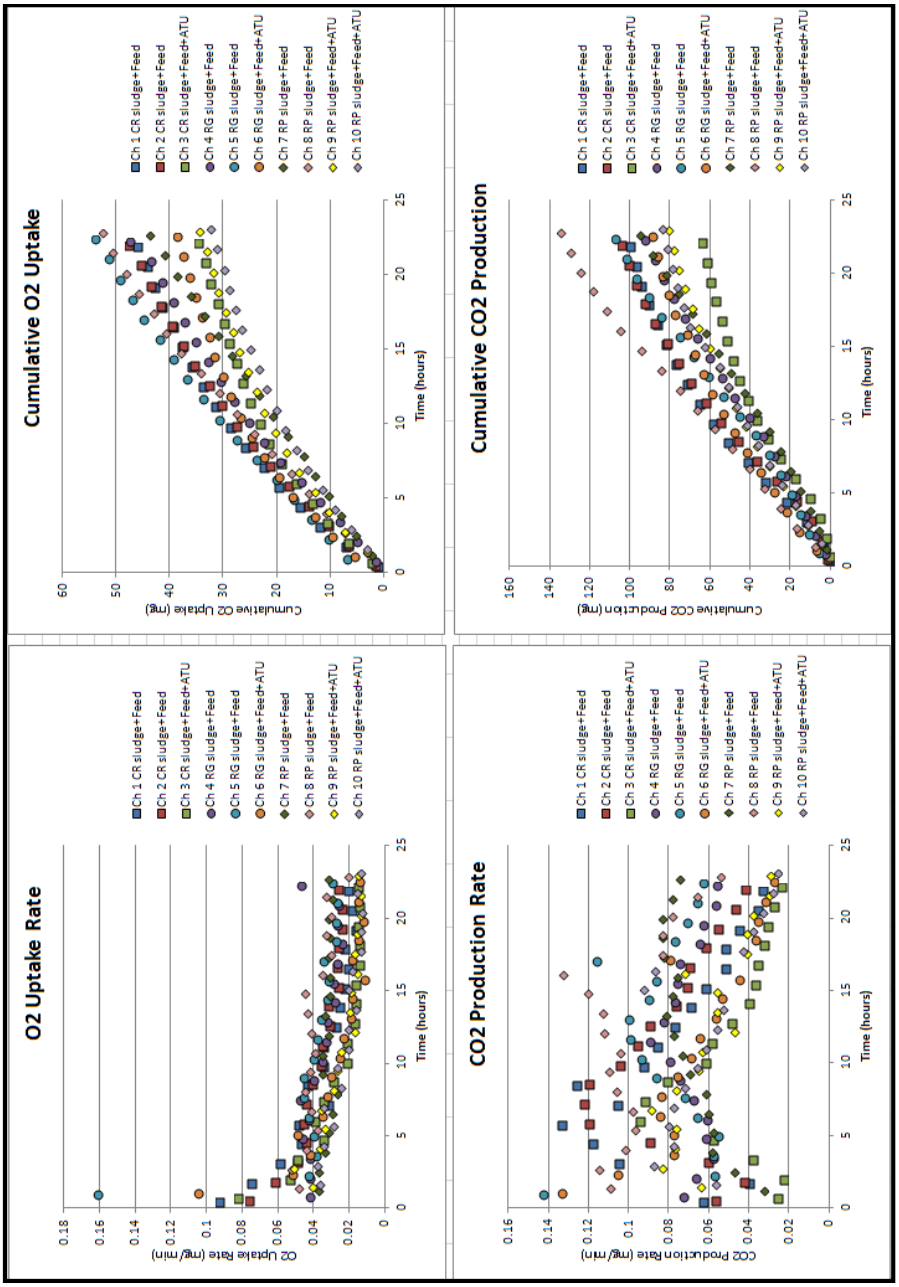

Figure B.8. Raw data of Test 8 (CR, RG, RP – 15.07.2014).

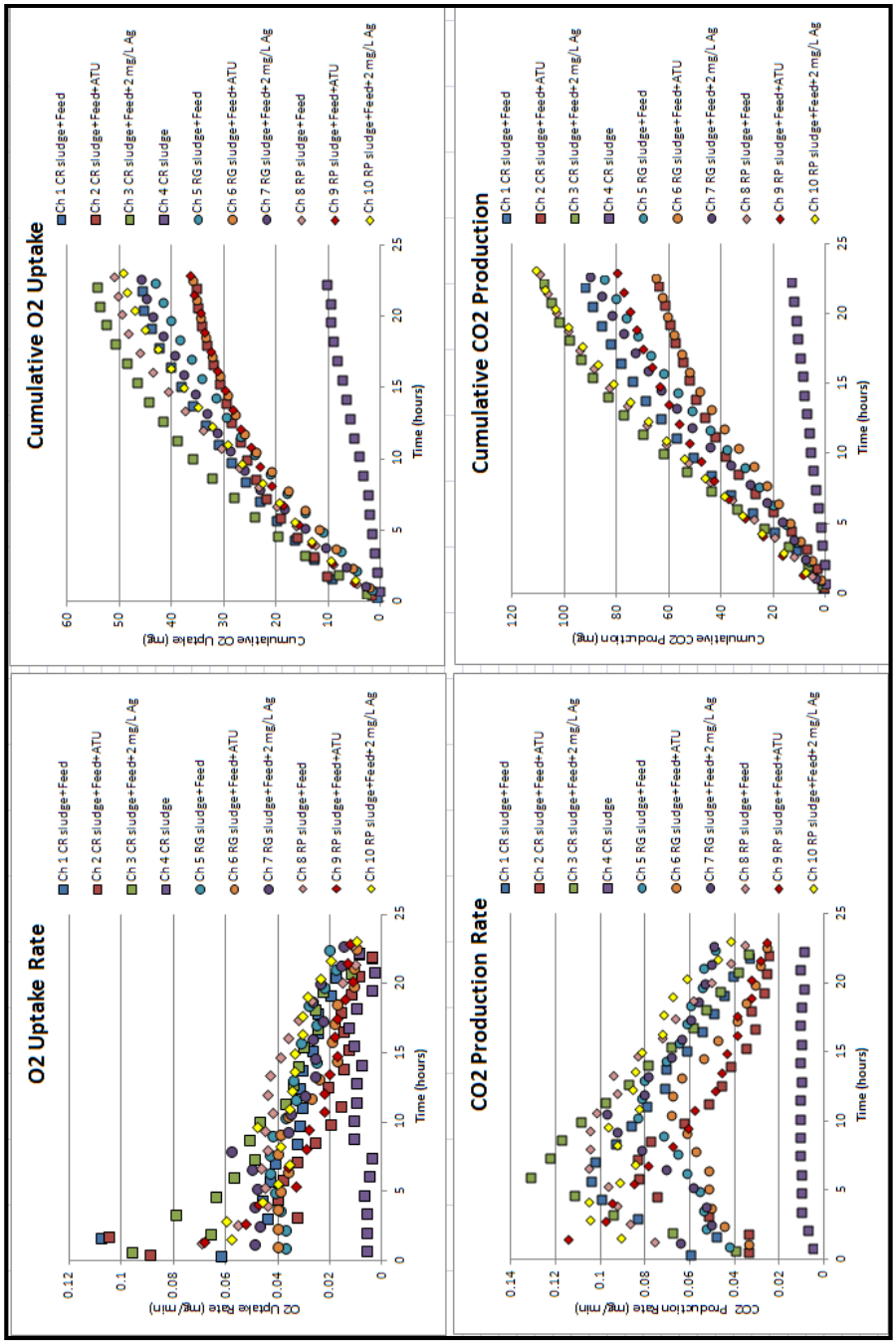

Figure B.9. Raw data of Test 9 (CR, RG, RP – 05.08.2014).

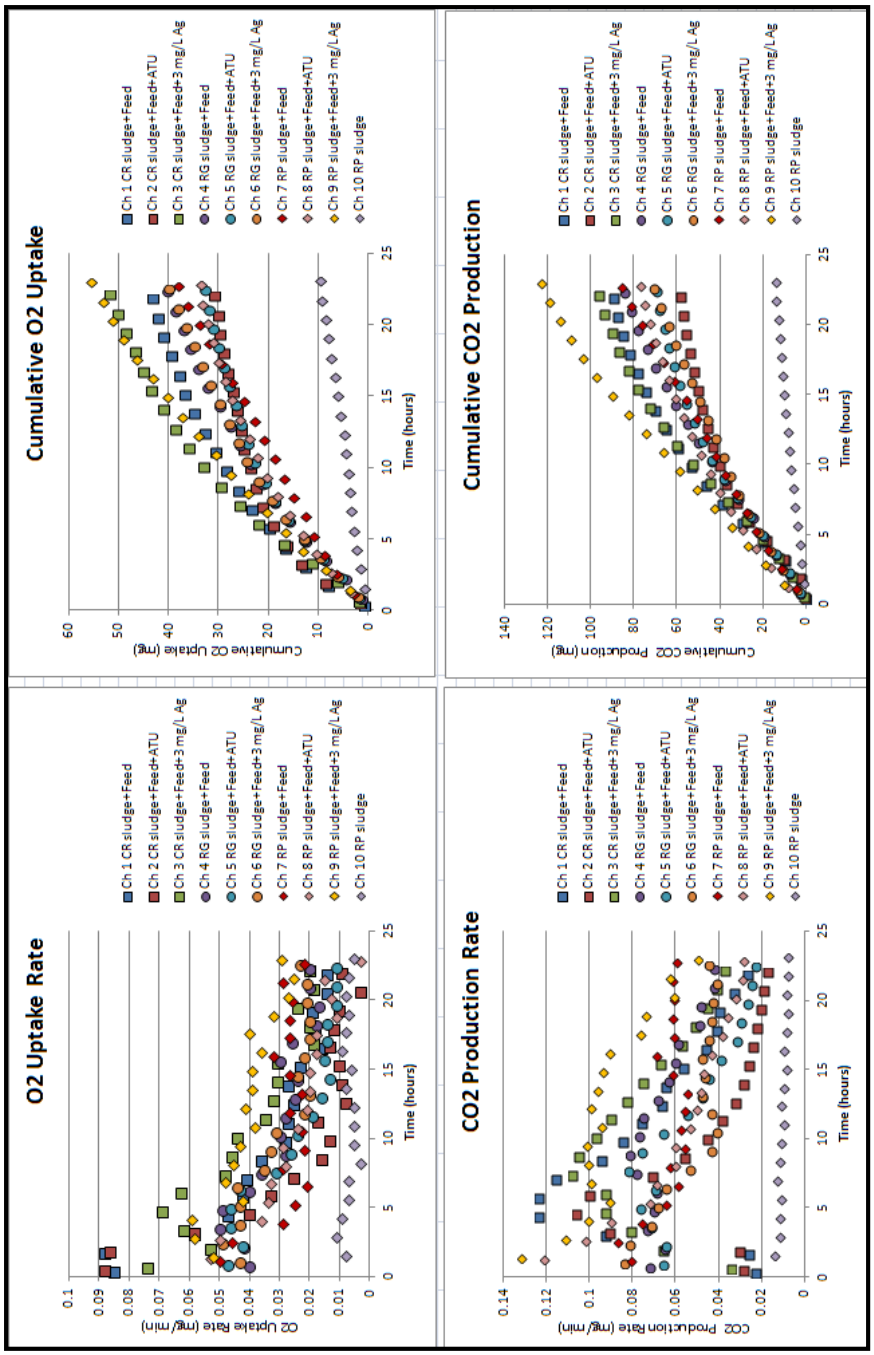

Figure B.10. Raw data of Test 10 (CR, RG, RP – 07.08.2014).

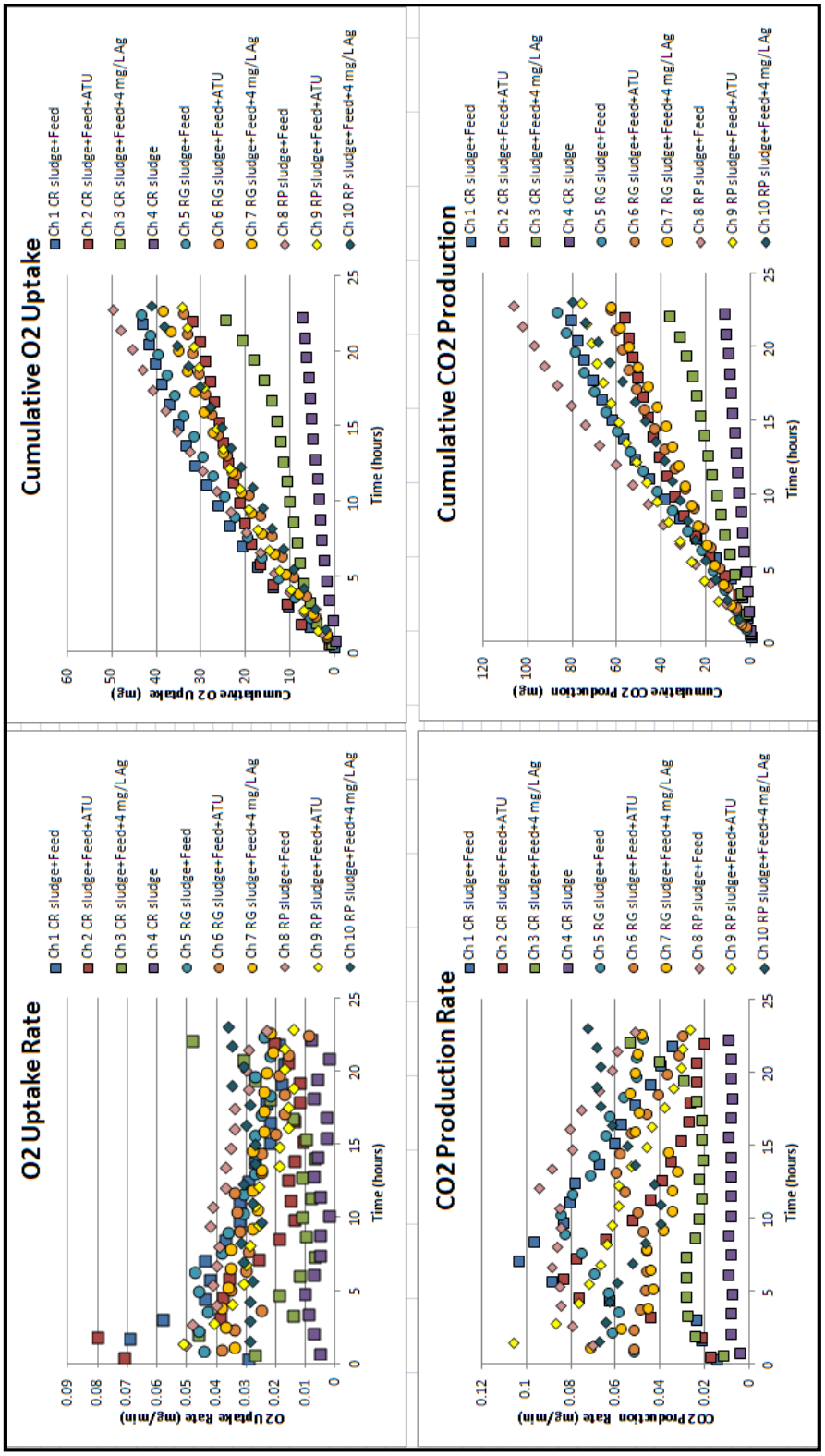

Figure B.11. Raw data of Test 11 (CR, RG, RP – 12.08.2014).

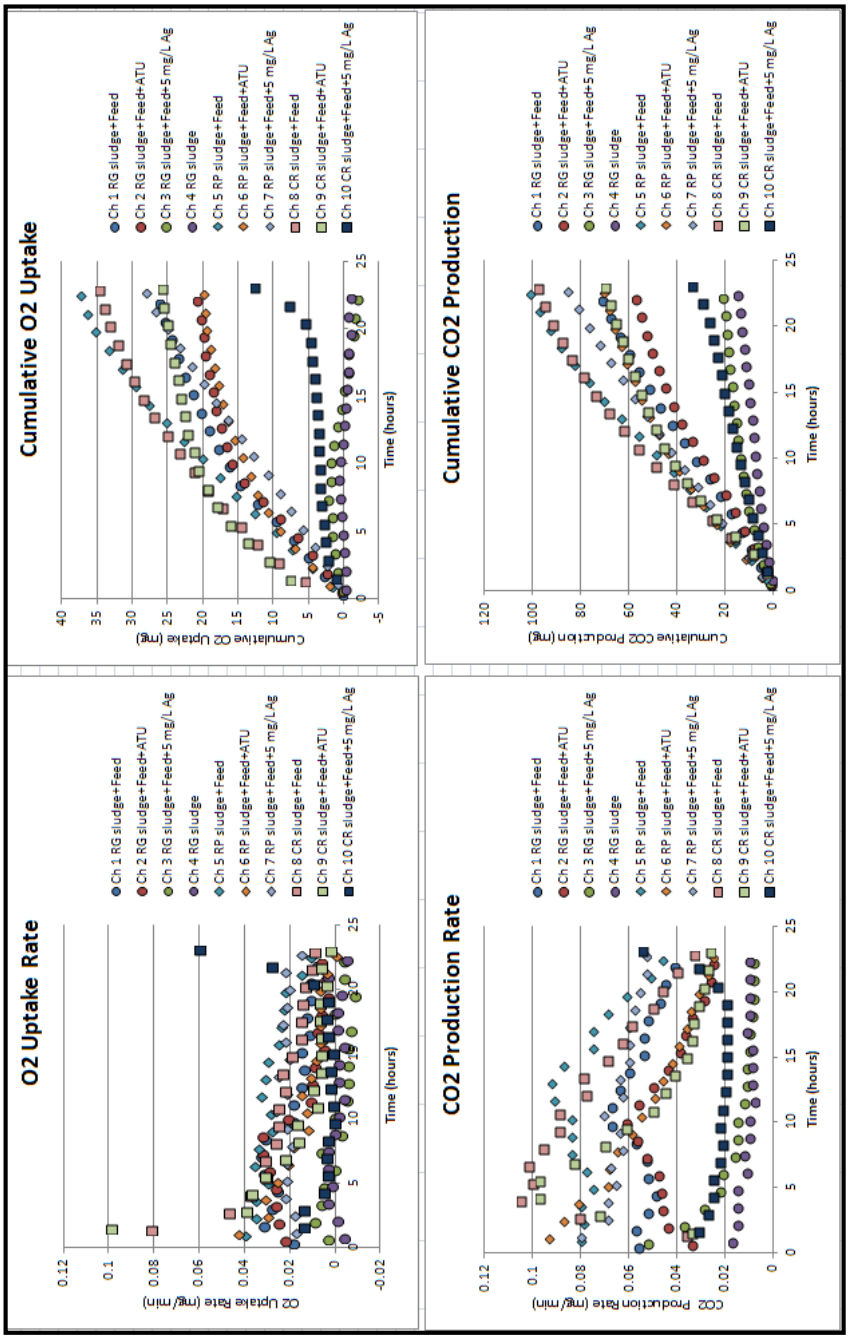

Figure B.12. Raw data of Test 12 (CR, RG, RP – 19.08.2014).

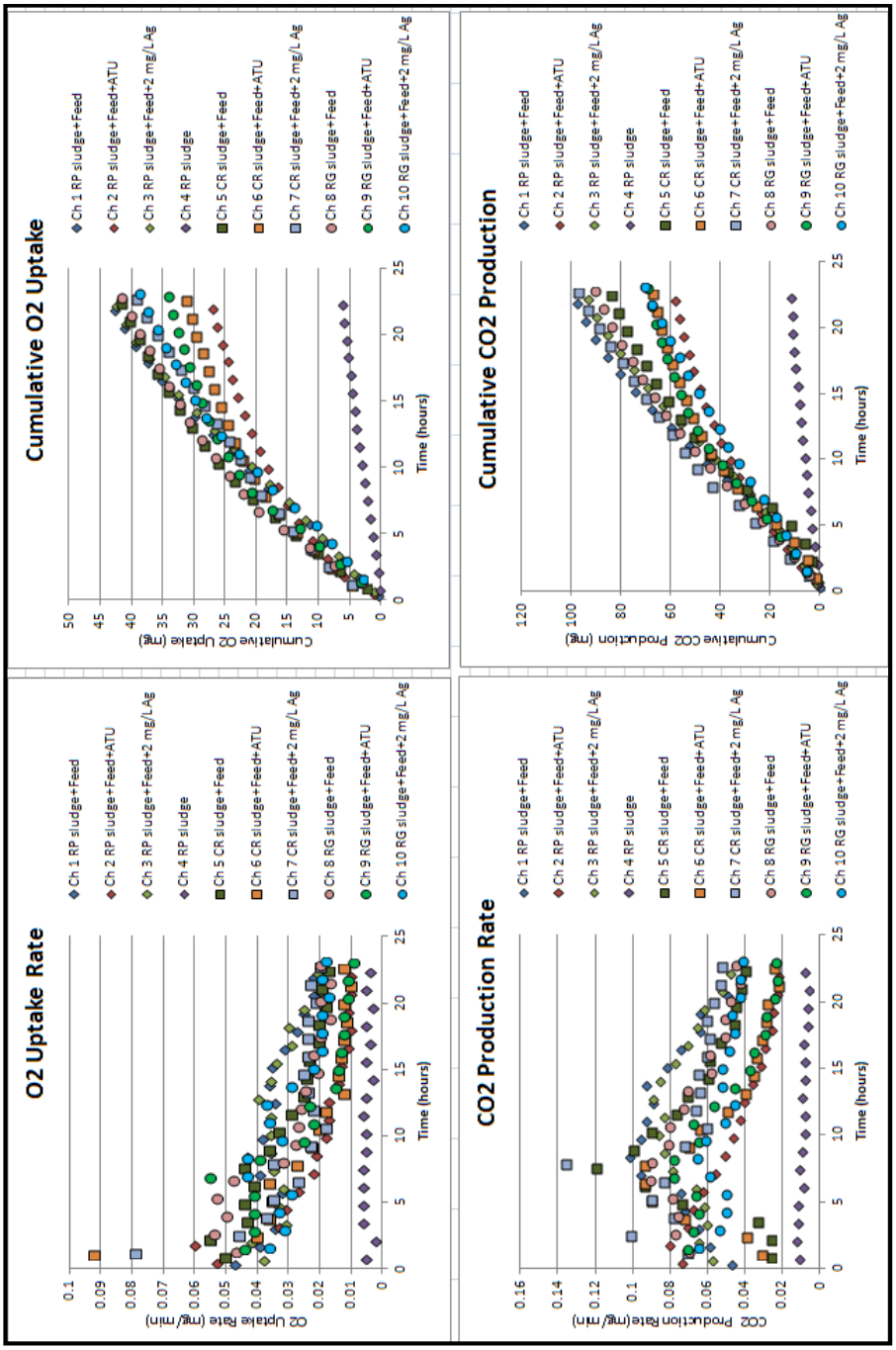

Figure B.13. Raw data of Test 13 (CR, RG, RP – 21.08.2014).

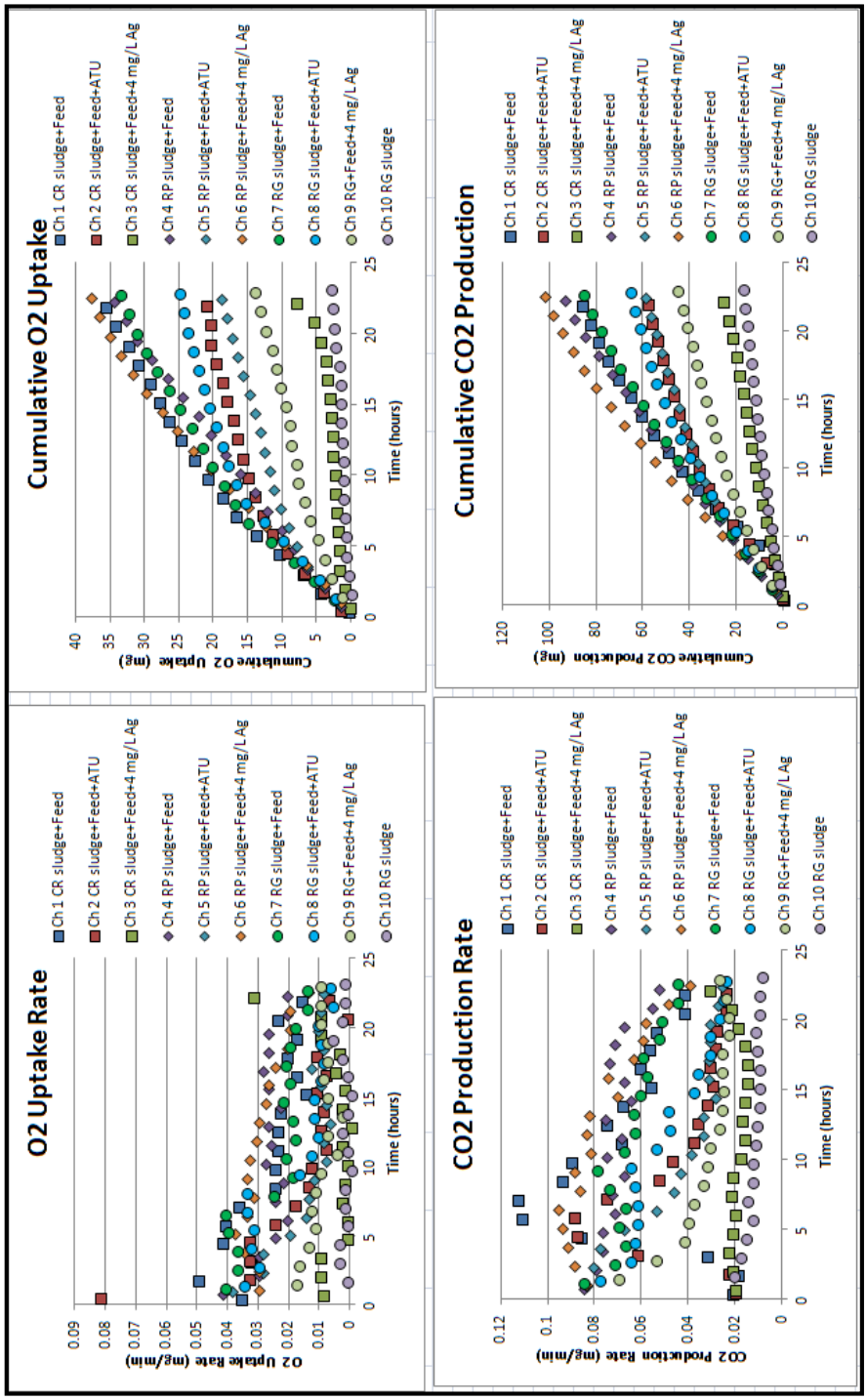

Figure B.14. Raw data of Test 14 (CR, RG, RP – 26.08.2014).

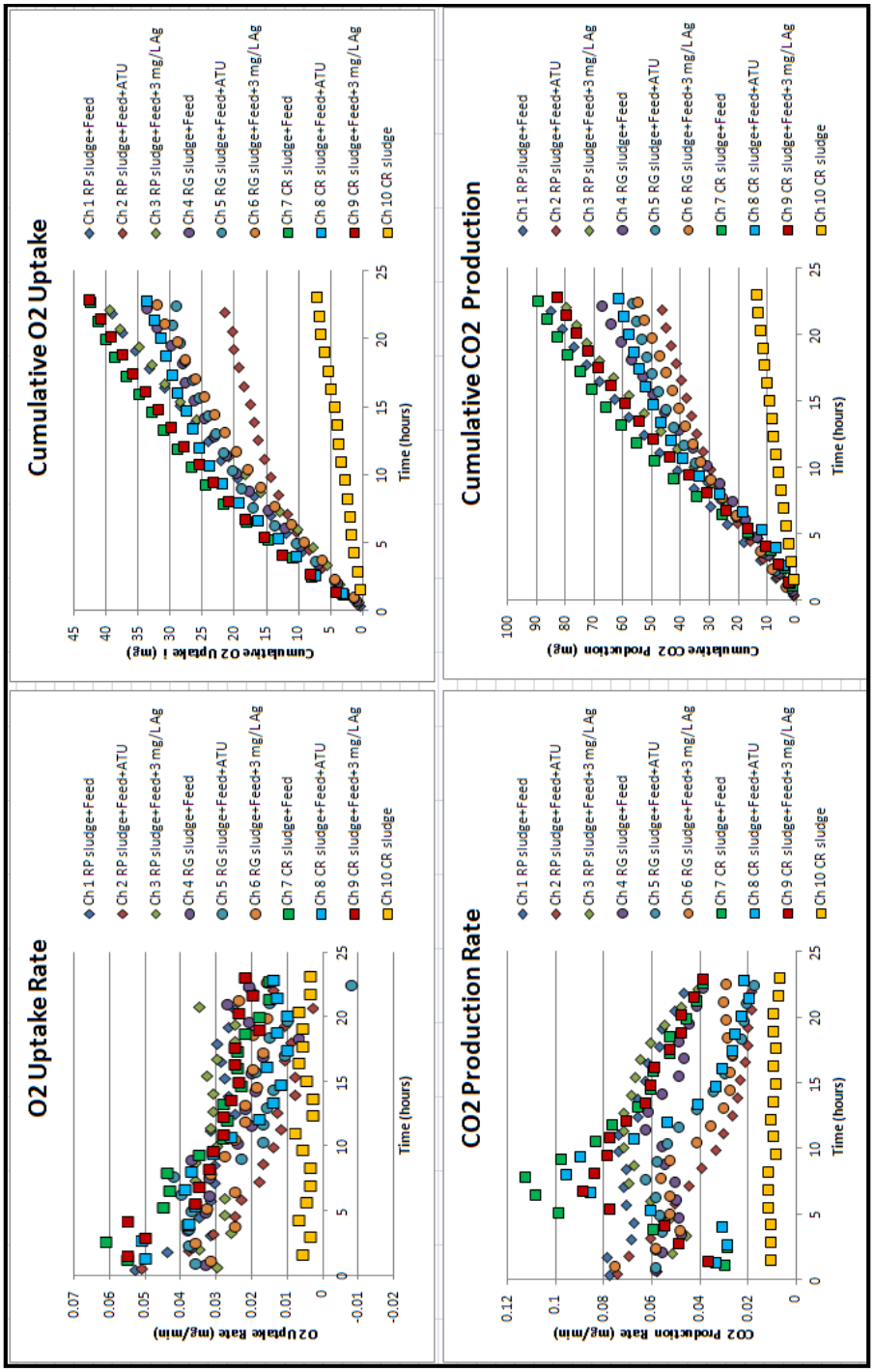

Figure B.15. Raw data of Test 15 (CR, RG, RP – 28.08.2014).

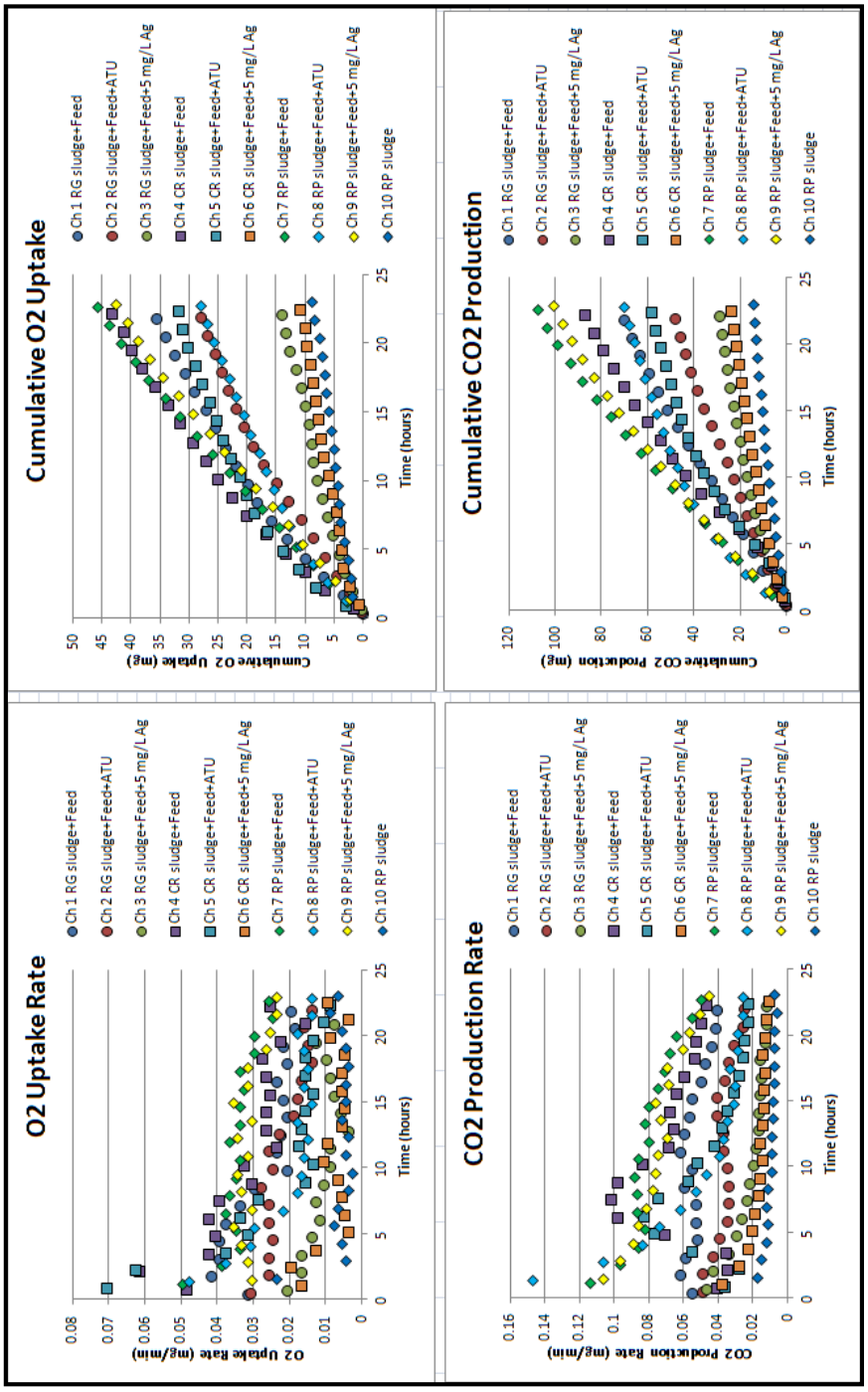

Figure B.16. Raw data of Test 16 (CR, RG, RP – 02.09.2014).

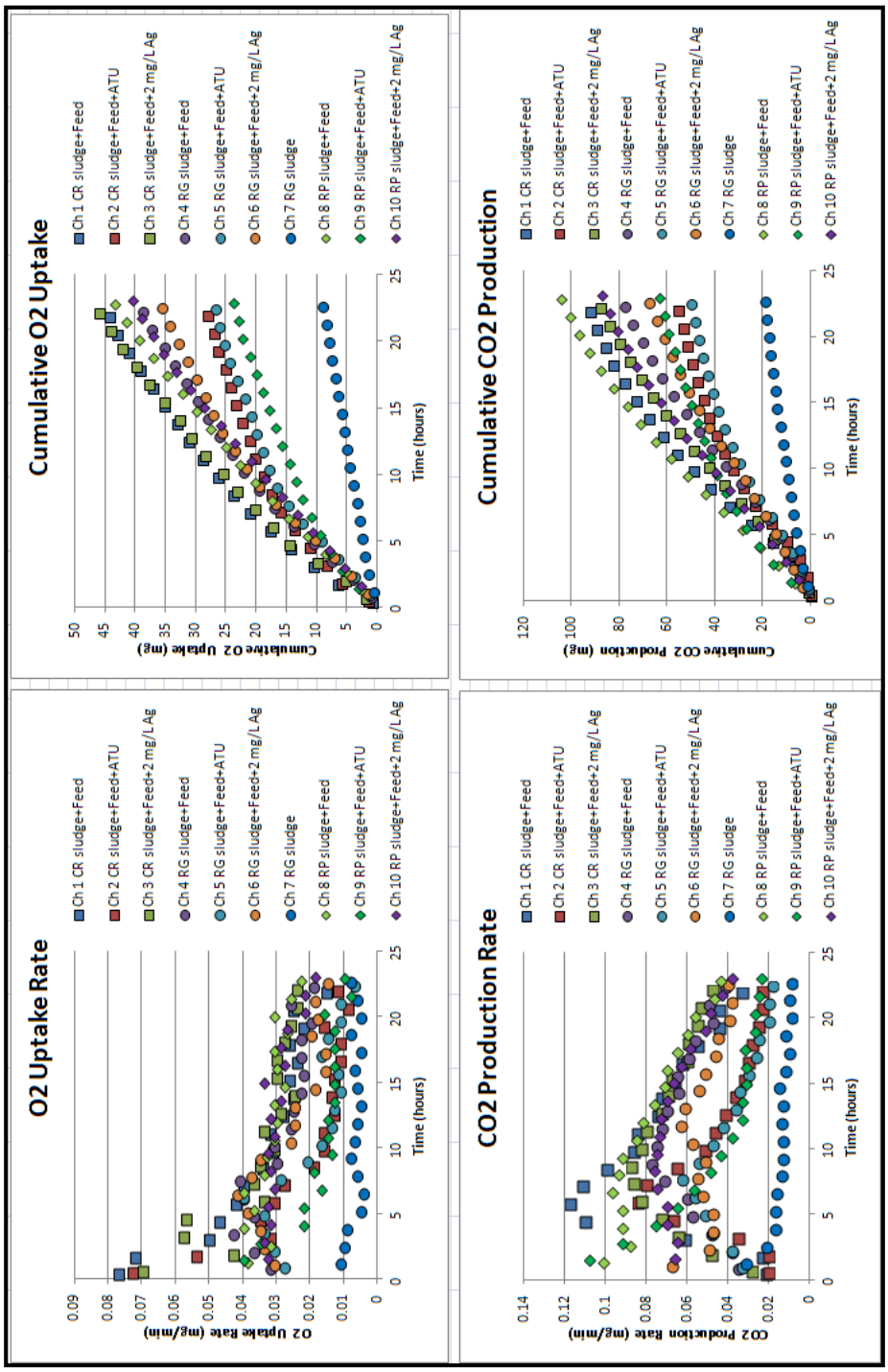

Figure B.17. Raw data of Test 17 (CR, RG, RP – 04.09.2014).

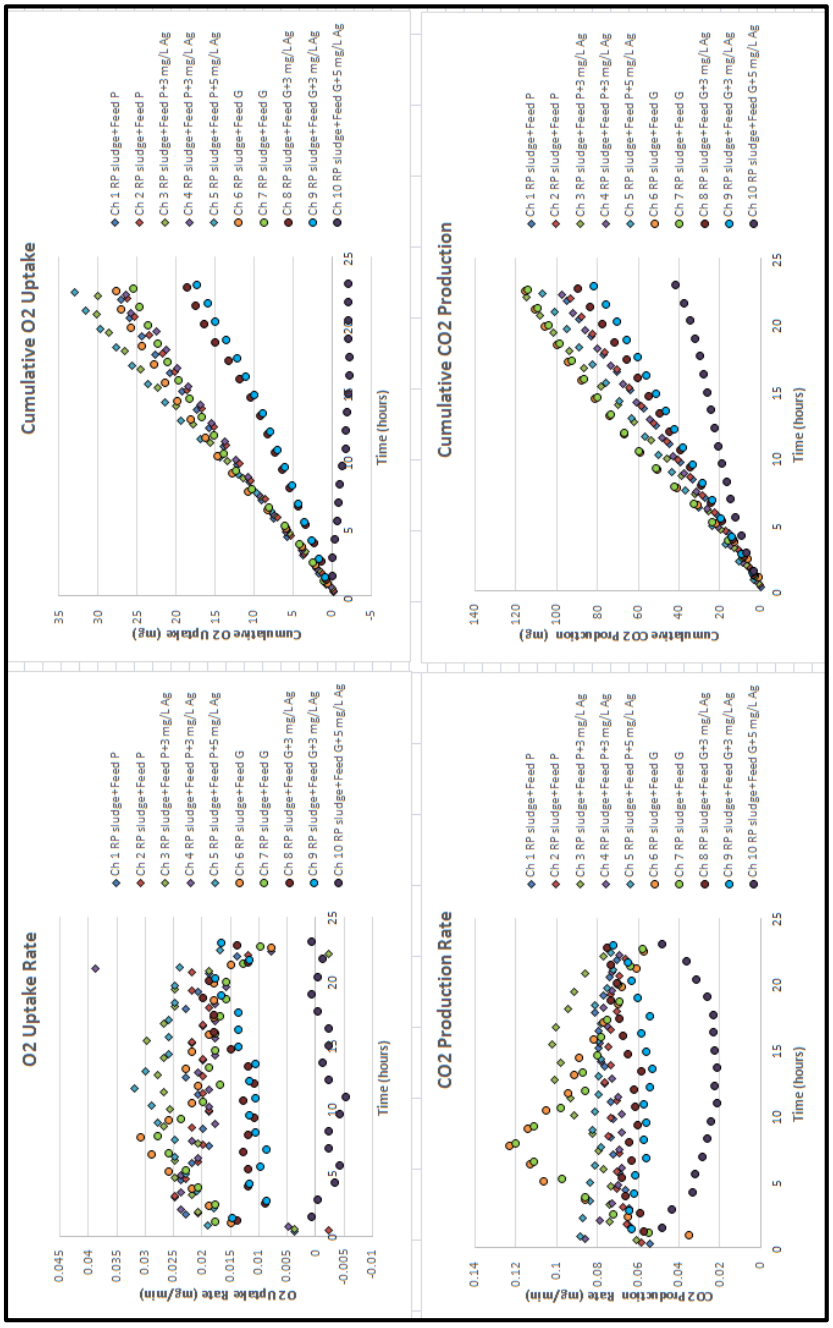

Figure B.18. Raw data of Test 18 (RP – 20.10.2014).

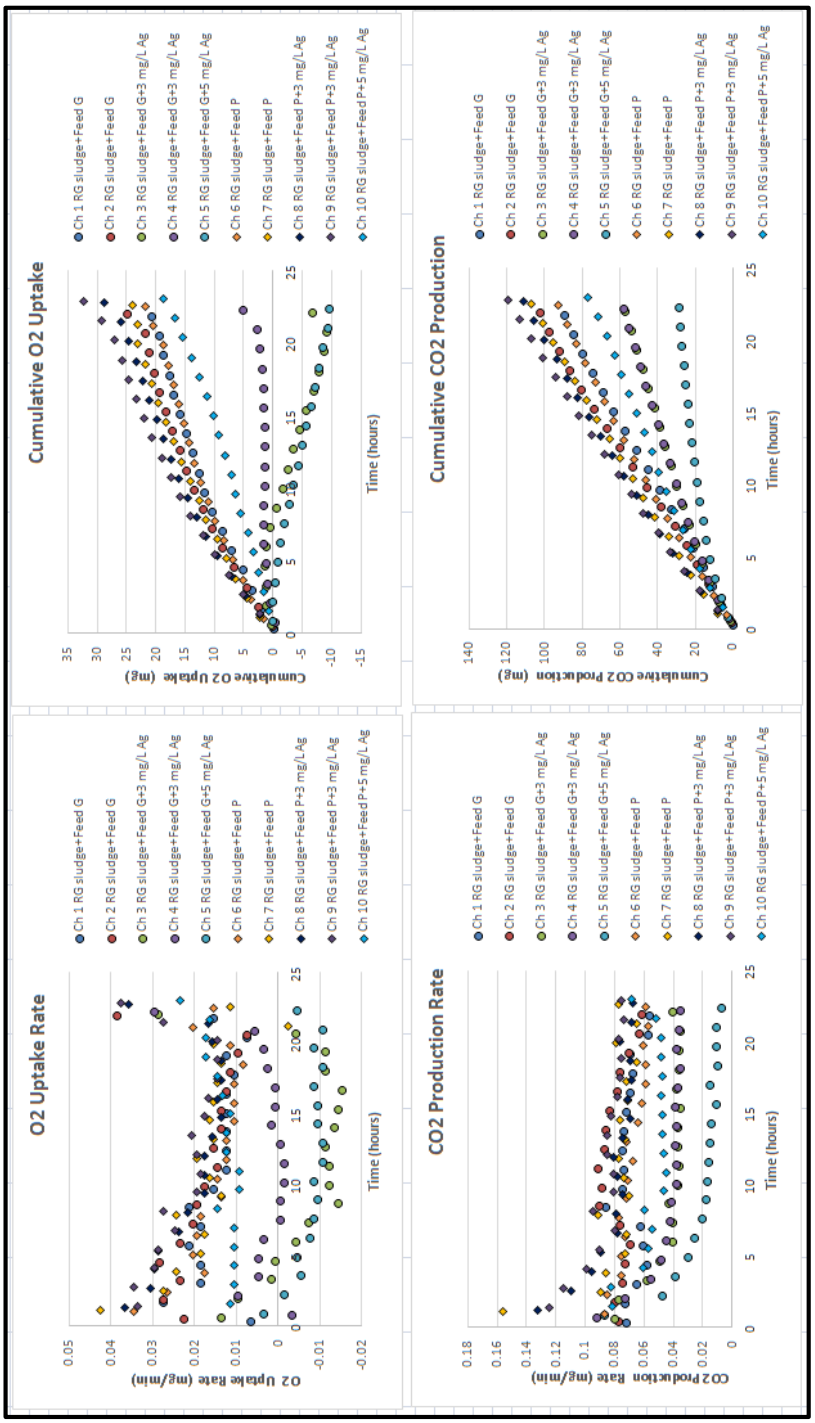

Figure B.19. Raw data of Test 19 (RG – 27.10.2014).

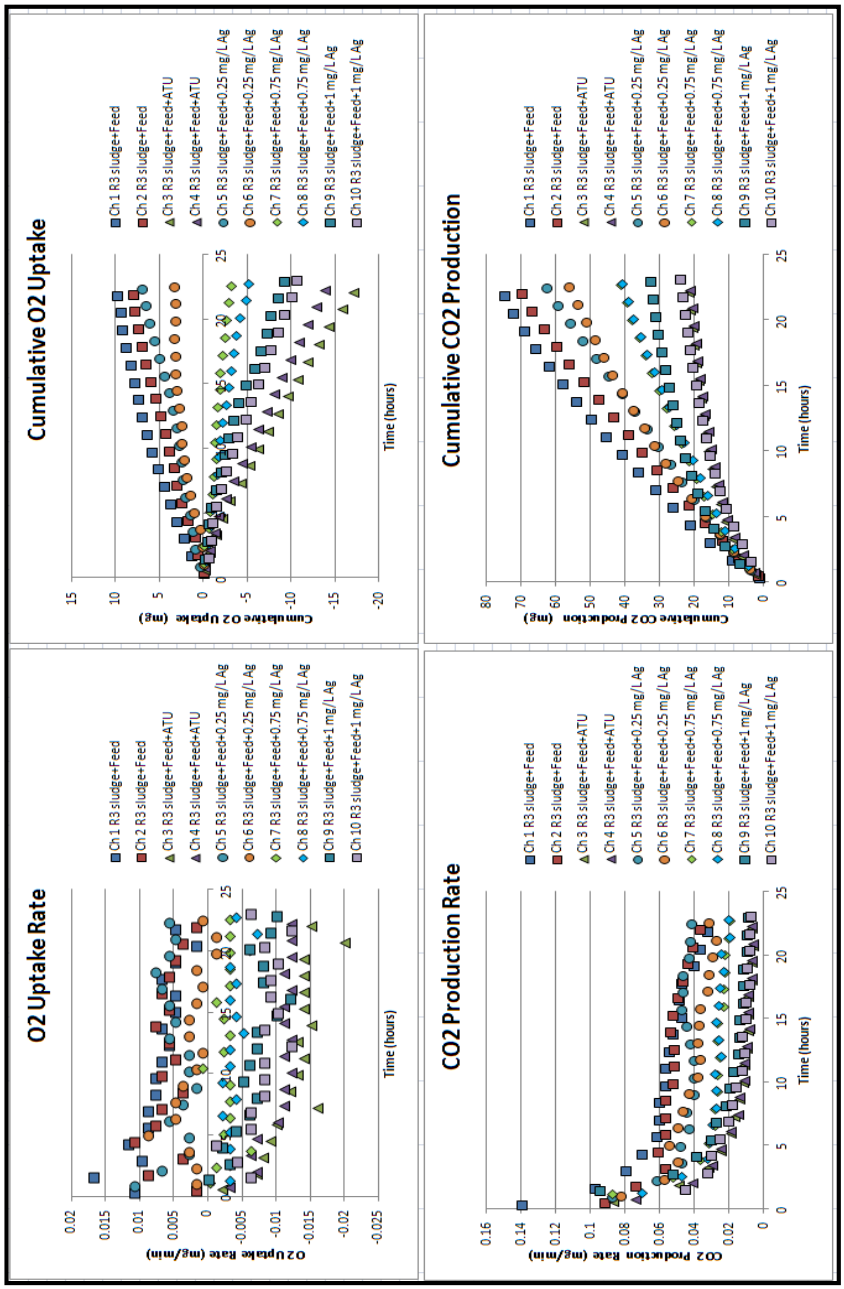

Figure B.20. Raw data of Test 20 (R3 – 10.11.2014).

APPENDIX C: ISOTHERMS OF SORPTION TESTS

Results of CR Reactor:

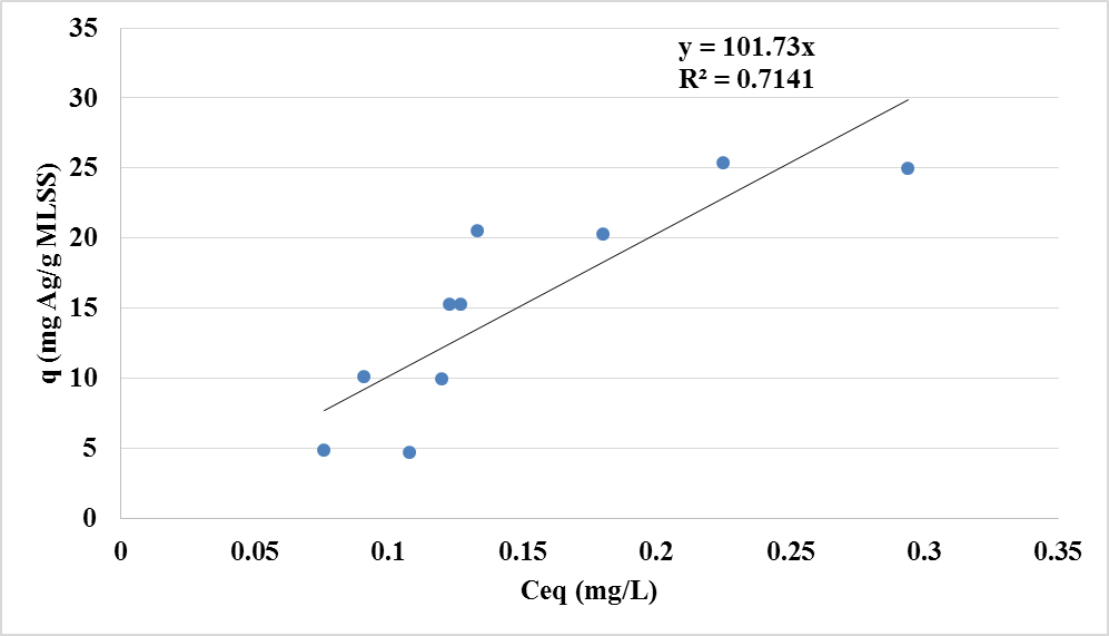

Figure C.1. Linear isotherm for the CR sludge.

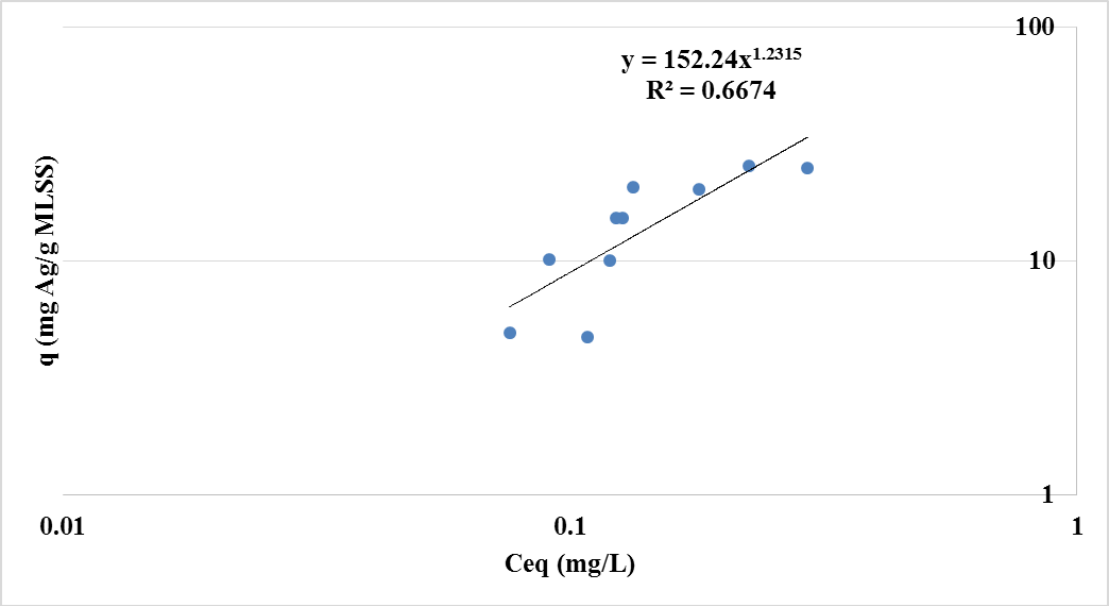

Figure C.2. Freundlich isotherm for the CR sludge.

Results of RG Reactor:

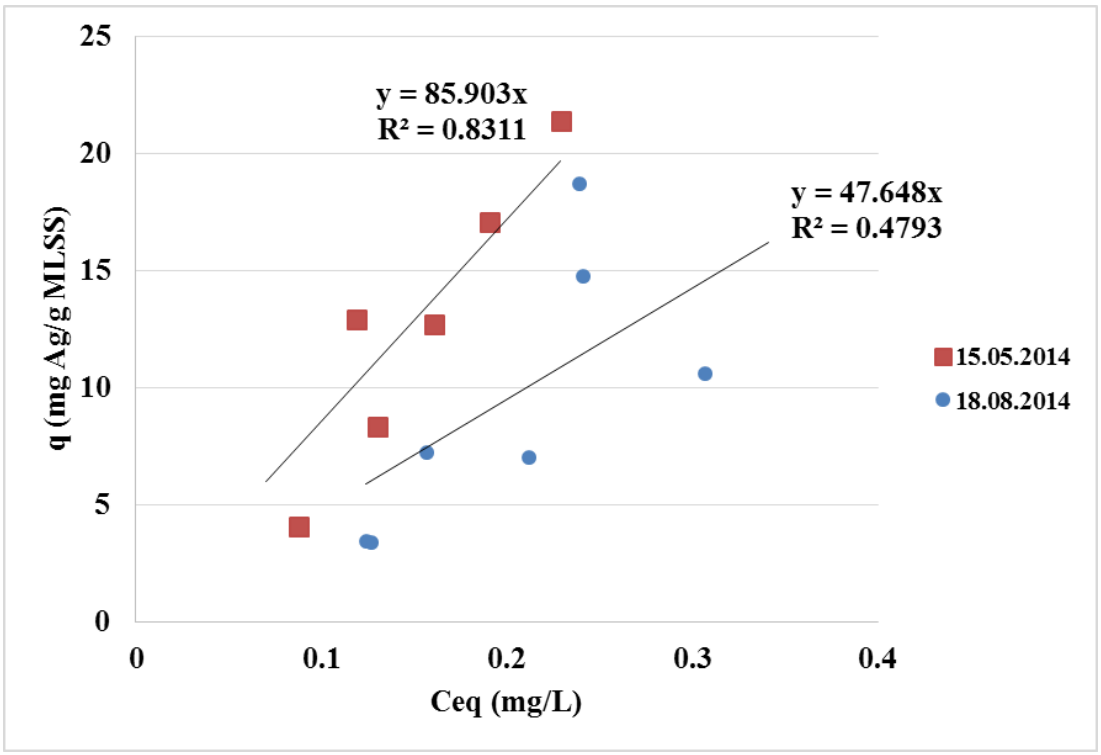

Figure C.3. Linear isotherm for the RG sludge.

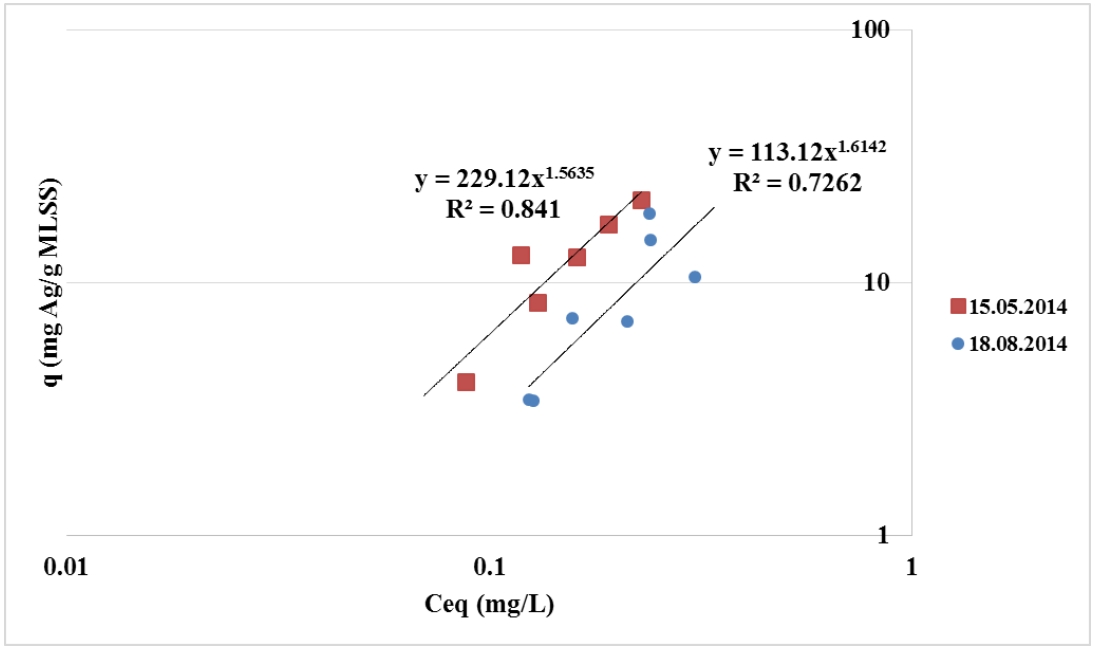

Figure C.4. Freundlich isotherm for the RG sludge.

Results of RP Reactor:

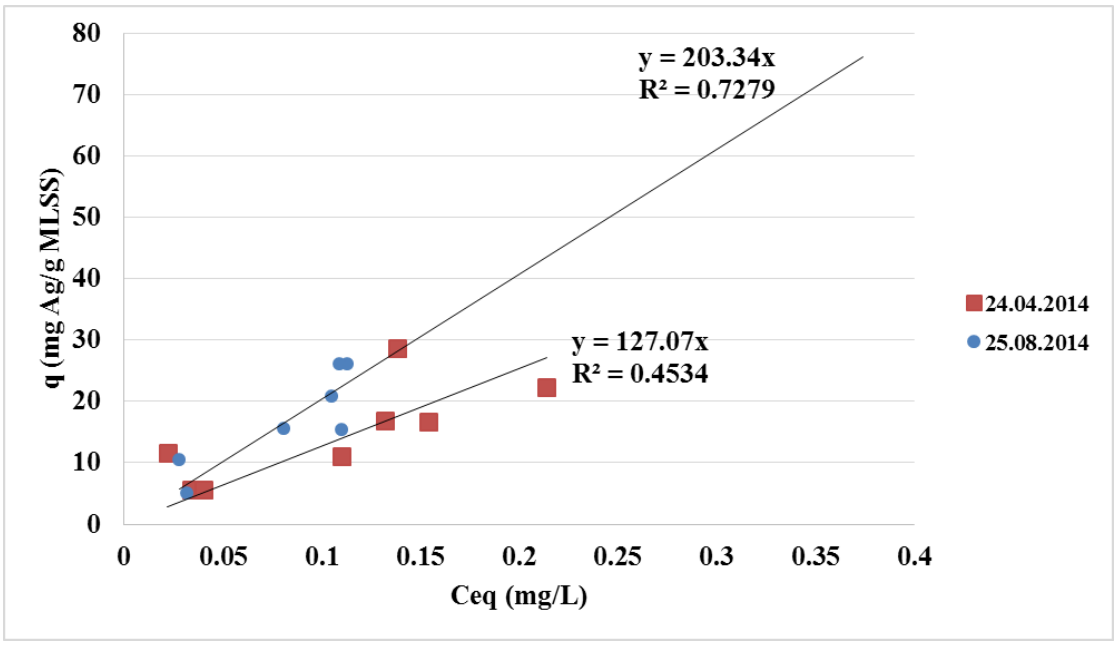

Figure C.5. Linear isotherm for the RP sludge.

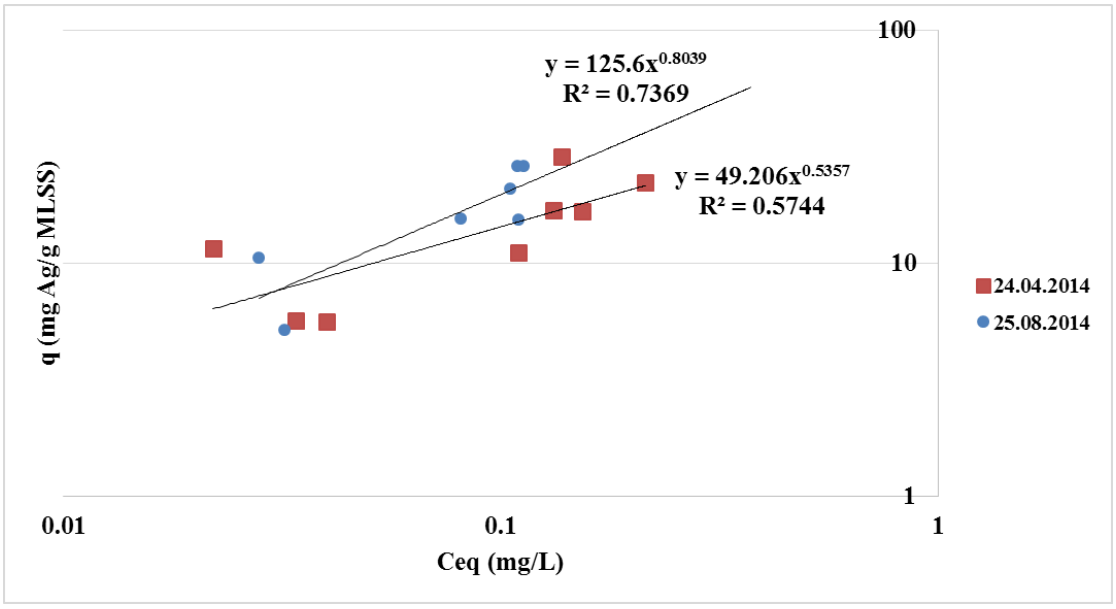

Figure C.6. Freundlich isotherm for the RP sludge.