MARMARA UNIVERSITY
INSTITUTE FOR GRADUATE STUDIES
IN PURE AND APPLIED SCIENCES

OPTIMIZATION OF OZONATION CONDITIONS FOR THE PHOSPHORUS RECOVERY FROM WASTE ACTIVATED SLUDGE

SEVİL COŞGUN

MASTER THESIS

Department of Environmental Engineering

Thesis Supervisor
Associate Prof. Dr. Neslihan Semerci

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ABSTRACT

Optimization of Ozonation Conditions for the Phosphorus Recovery from Waste Activated Sludge

This study focused on the determining the optimum ozonation conditions for phosphorus recovery from waste activated sludge. Twenty-six batch experiments were run with or without acidic and alkaline pre-treatments and different ozone dosages. Also, microwave post-treatments were applied to enhance phosphorus release efficiency in some experiments. Ozonation without any pre or post-treatment was observed as almost ineffective for phosphorus release. Among the different pH levels examined, most effective pre-treatment to enhance ozonation efficiency has determined as alkaline. However excessive ozonation with alkaline pre-treatment caused to lose of a part of released phosphorus, probably due to struvite or apatite precipitation at high pH levels. Acidic pre-treatment showed a negative effect on sludge solubilization during ozonation. But, phosphorus release during ozonation was enhanced by acid pre-treatment probably due to solubilization of inorganic particulate phosphorus in addition to cell lysis. For microwave treatment, acid pre-treated sludge showed significantly higher release than alkaline pre-treated or neutral sludge. Among different process combinations, acid pre-treatment/ozonation/microwave experiments have shown the highest sludge solubilization and phosphorus release. Also, both sludge samples from aeration tank and recycle line were defined as suitable for phosphorus recovery. Recovery of released phosphorus was an another critical point in this study. For struvite precipitation, pH:8.5 were determined as optimum level. Also using fine struvite particles as seed and relatively long crystallization time as two days increased struvite precipitation efficiency.

Keywords: phosphorus recovery, ozonation, microwave, struvite.

ÖZET

Atık Aktif Çamurdan Fosfor Gerikazanımı için Ozonlama Koşullarının Optimizasyonu

Bu çalışma atık aktif çamurdan fosfor geri kazanımı için optimum ozonlama koşullarının belirlenmesine odaklanmıştır. Asidik veya alkali ön işlemli ya da işlemsiz ve farklı ozon dozlarında 26 kesikli deney yapılmıştır. Ayrıca bazı deneylerde fosfor salınım verimini arttırmak için ozonlama sonrası mikrodalga işlemi uygulanmıştır. Başka bir işlem olmadan ozonlamanın fosfor salınımında neredeyse etkisiz olduğu gözlemlenmiştir. Denenen farklı pH değerleri içinde ozonlama verimini arttırmakta en etkili ön işlem alkali olarak belirlenmiştir. Ancak alkali ön işlemle uzun süre ozonlama, muhtemelen yüksek pH'larda struvit veya apatit çökmesine bağlı olarak, salınan fosforun bir kısmının kaybına neden olmuştur. Asidik ön işlem ozonlamayla çamur çözünmesinde negatif etki göstermiştir. Fakat ozonlama esnasında fosfor salınımı, muhtemelen hücre tahribine ek olarak inorganik fosfor partiküllerinin çözünmesiyle, asidik ön işlemle artmıştır. Mikrodalga için asidik ön işlemli çamur, alkali ön işlem görmüş veya nötr çamurdan oldukça daha fazla salınım göstermiştir. Farklı kombinasyonlar içinde asidik ön işlem/ozonlama/mikrodalga deneyleri en yüksek çamur çözünümü ve fosfor salınımını göstermiştir. Ayrıca, havalandırma tankı ve geri devir hattından alınan çamur örneklerinin ikisinin de fosfor geri kazanımı için uygun olduğu belirlenmiştir. Salınan fosforun gerikazanımı bu çalışmanın başka bir kritik noktasını oluşturmaktadır. Struvit çöktürmesi için pH:8,5 optimum koşul olarak belirlenmiştir. Ayrıca ince struvit partiküllerinin çekirdek olarak kullanımı ve iki gün gibi kısmen uzun bir kristalizasyon süresi struvit çöktürme verimini arttırmıştır.

Anahtar kelimeler: fosfor gerikazanımı, ozonlama, mikrodalga, strüvit.

SYMBOLS

°C: Centigrade degrees

°K: Kelvin degrees

ΔH°: Enthalpy

Pa: Pascal

Atm: Atmosphere (pressure)

Mhz: Megahertz

J: Joule

kJ: Kilojoule

cal: Calorie

V: Volt

d: Day

hr: Hour

min: Minute

s: Second

rpm: Rotation per minute

L: Liter

ml: Mililiter

g: Gram

mg: Miligram

%: Percentage

ABBREVIATIONS

AAS: Atomic Absorbsion Spectrometry

AHP: Acid hydrolyzable phosphorus

AP: Apatite phosphorus

Ca: Calcium

FISH: Fluorescence In-Situ Hybridization

IP: Inorganic phosphorus

Mg: Magnesium

MLSS: Mixed Liquor Suspended Solid

MLVSS: Mixed Liquor Volatile Suspended Solid

MW: Microwave

NAIP: Non-apatite phosphorus

NH_4-N: Ammonia nitrogen

NO_3-N: Nitrate nitrogen

O_2: Oxygen

O_3: Ozone

ORG-P: Organic phosphorus

pH: Power of Hydrogen

PO_4-P: Reactive phosphorus

sCOD: Soluble Chemical Oxygen Demand

SMT: Standards, measurements and testing protocol.

tCOD: Total Chemical Oxygen Demand

TP: Total phosphorus

WWTP: Wastewater treatment plant

Zn: Zinc

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1. INTRODUCTION

Biological wastewater treatment can be basically defined as degradation of suspended or dissolved materials in wastewater by bacteria. As the most common method of biological wastewater treatment, activated system has been used for treating numerous kinds of wastewater and over 90% of municipal wastewater treatment plants have employed activated sludge systems or its modifications (Liu,2003). Biological wastewater treatment depends on converting organics and nutrients to carbon dioxide, water and bacterial cells. To attain the desired bacterial cell concentration in the system, part of produced sludge, namely excess sludge, must be withdrawn and be disposed of safely and cost effectively in concentrated form named as excess sludge. The disposed excess sludge reaches excessive amounts, 10 million tons of dry solids in the European Union, 8 million tons of dry solids in the United States, and 11 million tons of dry solids in China in 2010 (Paul and Liu, 2012). Disposal of waste activated sludge is one of the most expensive problems in wastewater treatment plants, the treatment of the excess sludge may account for up to 60% of the total plant operation cost (Zhang et al., 2017).

Another significance of activated sludge is its phosphorus rich content as a result of phosphorus accumulating organisms. Phosphorus is an essential and nonreplaceable element for all the living. Considering the phosphorus depletion, one of the ideal ways to solve sludge-associated problems is the phosphorus recovery. However, disintegration of bacterial cell for the release of phosphorus is the most challenging step. It should be efficient, economical and applicable. Numerous sludge disintegration technologies based on thermal, chemical, biological and mechanical processes and their combinations can be applied for solubilization and lysis. Ultrasonic treatment, ozonation, mechanical disintegration, alkaline treatment, thermal treatment, microwave and biological processes are some of the investigation objectives studied by several researchers in lab-scale plants (Erden et al., 2010).

This work deals with the optimization of ozone oxidation in terms of the effect of phosphorus solubility in the excess sludge.

1.1. Sludge Disintegration

Sludge disintegration refers to technologies to break sludge flocs, enhance hydrolysis and microbial decomposition by rupturing cell wall and causing cell lysis. Activated sludge involves flocs which consist bacteria, algae, archea and fungi bounded by extracellular polymeric substances (EPS) (Guo and Yu-Feng, 2011). EPS are a cocktail of carbohydrates, lipids, nucleic acids, proteins, and humic acids (Ding et al., 2017). To date, several sludge disintegration methods have been developed and applied due to prevalence of activated sludge systems for biological wastewater treatment. Chemical, biological and physical disintegration are three prominent processes which require chemical or enzymatic additions or heat, pressure, shear force applications (Wett et al.,2010). While the thermal, chemical or biological processes basically break the protein-lipid bilayer of cell and releases the intracellular substances, mechanical methods extensively disintegrate the sludge like EPS solubilization (Liu et al.,2017). Enzyme treatment, mechanical treatment, ozonation, chemical treatment, thermal treatment, microwave and ultrasound are some of the distinguished disintegration technologies. Also, combinatinos of these processes may be applied to the sludge for enhanced disintegration.

Sludge disintegration can be used for different applications such as sludge reduction, improved stabilization, recovery as a carbon source, recovery of phosphprus and nitrogen, pathogen reduction, and improved settling, conditioning and dewatering properties (Müller, 2003).

The term of disintegration may be confused with degradation, which is basically the reduction of organic material (Vranitzky and Lahnsteiner, 2005).

1.1.1. Enzymatic hydrolysis

Complex organics and floc structures in the activated sludge can be disintegrated by extracellular enzymes, including protease, amylase, and lipase enzymes (Burgess and Pletschke, 2008). Cell lysis (which means destruction of cells, releasing intracellular components and disintegration of insoluble organic particles to smaller carbonhydrates) can be catalyzed hydrolytic enzymes (Guo and Yu-Feng, 2011). Hydrolytic enzymes also breaks EPS by a multistep process, initially adsorbing the sludge-substrate, attacking the polymeric substance, forming enzyme substrate complexes, hydrolyzation

of small polymers and finally solibilisation. Enhanced filtering, dewatering and settling properties in sludge can be obtained by enzyme addition. (Foladori et al.,2010). Protein degradation also catalyzed by hydrolytic enzymes, breaking the protein down into peptides and aminoacids, and conversion of aminoacids into some low-molecular weight organic acids, ammonia, and carbon dioxide (Guo and Yu-Feng, 2011).

Commercial hydrolytic enzymes (protease, lipase, cellulase, hemicellulase, glycosidic) can be used to enhance sludge hydrolysis. But high cost of enzyme preparation is a important disadvantage of this process. So, bioaugmentation by enhancement of microorganisms or endogenous enzymes from the municipal sludge extracellular hydrolases, and inoculation of the extraneous enzyme producing strains could be feasible alternatives (Yu et al., 2013).

50°C is considered as the optimal temperature for hydrolitic enzyme activity on sludge solubilization. Thus, the enzymatic hydrolysis can be configurated with anaerobic digestion in mesophilic or thermophilic conditions successfully (Foladori et al.,2010).

1.1.2. Alkaline treatment

Alkaline treatment is a common method due to capability for disintegration of particulate organics at high pH and making the cellular substances more susceptible to reaction(Xiang,2017). High pH levels effect the bacterial cell wall in several ways including saponification of membrane lipids or negative charging of cell surface and desorption of some EPS due to high electrostatic repulsion (Neyens et al.,2003). While both alkaline hydrolysis and fermentation are applicable methods for sludge disintegration, studies have been mainly focused on the fermentation in the literature due to higher efficiencies. The study of Bi et al. (2014) has achieved 41.96% and 7.78% release efficiencies of PO_4-P and NH_4-N by alkaline hydrolysis while these figures were 83% and 75% in the alkaline fermentation study of Tong et al. (2009).

Effectivenes of alkaline treatment may be enhanced by combining with other technologies. Thermal-alkaline, alkaline-ultrasonic and microwave-alkaline pre-treatments are some of the most reported processes for sludge disintegration in the literature (Zahedi et al.,2017, Kim et al., 2015, Doğan and Sanin, 2009).

1.1.2.1. Thermal-alkaline treatment

Alkaline treatment becomes more effective when combined with thermal treatment. Zhang et al. (2009) had studied mesophilic and thermophilic hydrolysis at different pH levels between 4-11 and obtained the best results at pH:11. On the other hand, Kim et al. (2015) applied the same NaOH dosages at 25ºC and 50-80ºC and observed siginificantly higher phosphorus release at higher temperatures.

1.1.2.2. Ultrasonic-alkaline treatment

Ultrasound is an effective method due to decomposition effect on bacterial cells and facilitates the sludge dewatering. By combining ultrasound with alkaline treatment, the efficiency might be enhanced (Xiang et al., 2017). Park et al. (2012) had applied this combination as a pre-treatment for biogas production and obtained up to 5 times higher sludge solubilization.

1.1.2.3. Microwave-alkaline treatment

Microwave (MW) irradiation is an up and coming technology for sludge disintegration due to its disrupting effect on floc structures in waste activated sludge (Wang et al.,2016). Kennedy et al. (2007) applied combined MW hybrid alkaline treatment and obtained higher sludge solubilization as 21.7% than both MW (7%) and alkaline treatments (16.3%) (Tyagi et al.,2013). These might be the evidence of the synergistic effect of these two individually effective methods.

1.1.3. Ultrasonic disintegration

Ultrasound is an another current sludge disintegration technology by reducing floc size, disrupting microbial cells and enhancing sludge biodegradability (Tian et al., 2015). As shown in Figure 1.1, the black flocculent structure of sludge was dispersed after ultrasonic irradiation (Gong et al., 2015). Ultrasound pressure causes spreading waves through water, generating and crouching of gas bubbles strongly and rapidly. The shear force which led by this mobilization might be high as much as 5000 ºK temperature and 10^8 Pa. This phenomenon is named as acoustic cavitation (Huan et al., 2009). Xu et al. (2010) applied ultrasound for sludge disintegration and obtained a siginificant increase in sCOD concentration.

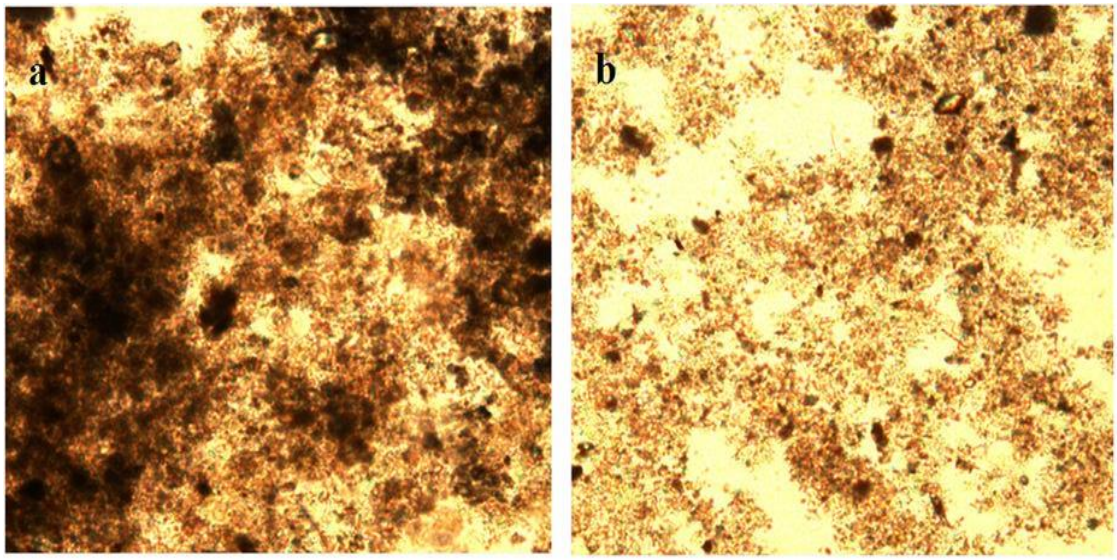


Figure 1. 1 Fluorescence microscopy images of sludge before(a) and after(b) ultrasound treatment (Gong et al., 2015).

1.1.4. Mechanical disintegration

As an another disintegration method, mechanical shear and pressure may be applied to the sludge to destroy flocs and and cell membrane. Mechanical disintegration in a well-known process in bioengineering to obtain enzymes and proteins as product (Müller, 2003).

Mechanical disintegration is mostly used for sludge handling because of being more suitable for high concentrated sludges. By reducing floc size, it increases the bacteria and substrate contact areas and enhanced the biodegradability. Mechanical disintegration might be a pre-treatment for anaerobic digestion. Also, there are some studies which proposes the mechanical disintegration as a solution for foaming problems due to fillamentus bacteria in anaerobic digesters (Foladori et al., 2010)

1.1.5. Thermal treatment

Thermal treatment is an effective method to improve microbial and organic degradation by sludge hydrolysis, EPS release and viscosity reduction (Yao et al., 2016 and Wang et al., 2017). Up to 63% COD solubilization and 235% VS reduction have been reported in the literature by thermal treatment (Pilli et al., 2015). Thermal hydrolysis is a well-known and probably the most effective pre-treatment for enhanced anaerobic digestion due to energy balance (Sapkaite et al., 2017). Consequently, lots of established and licenced systems have been applied in industrial scale like Cambi®, Biothelys®,

Lysotherm®, TPH®, Turbotec®, Exelys® (Sapkaite et al., 2017), schematic flow diagram of the Cambi®, the most known system among these, might seen in Figure 1.2.

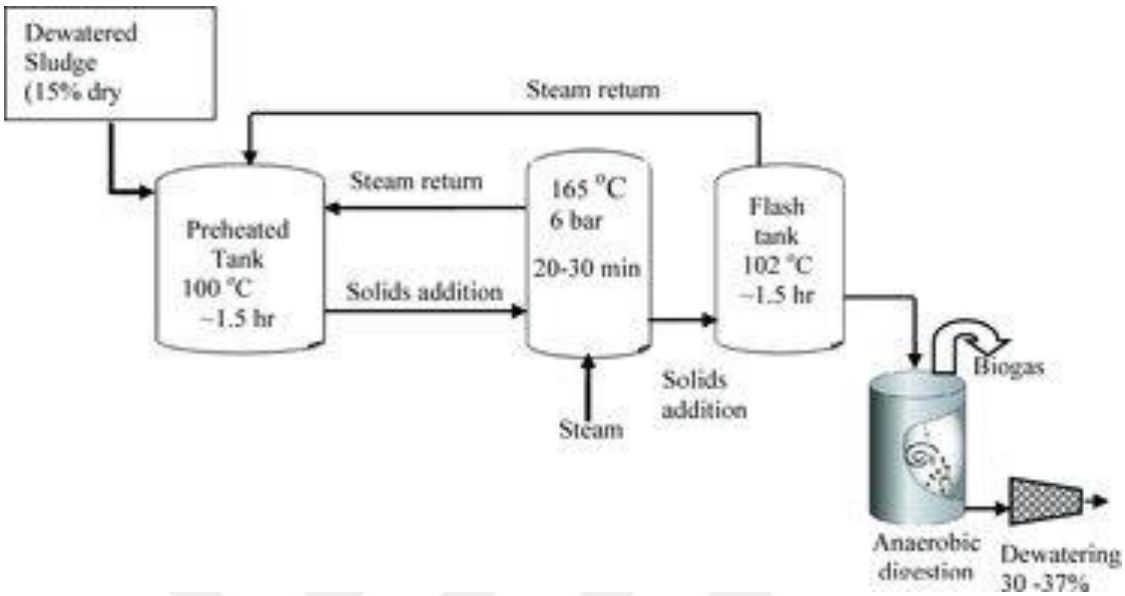


Figure 1. 2. Flow diagram of Cambi® process (Pilli et al., 2015).

1.1.6. Microwave irradiation

Microwave irradiation is a promising technology for different environmental applications like soil, wastewater and activated sludge treatment. Microwave treatment of sludge might be applied for different objectives like sludge disintegration, nutrient recovery, metal stabilization and sludge sterilization (Tyagi et al., 2013).

As shown in Figure 1.3, microwave refers to the energy on the electromagnetic spectrum between 300 and 300,000 MHz, between infrared and radiowaves an equivalent to 1 cm to 1 m wavelengths (Kostas et al., 2017).

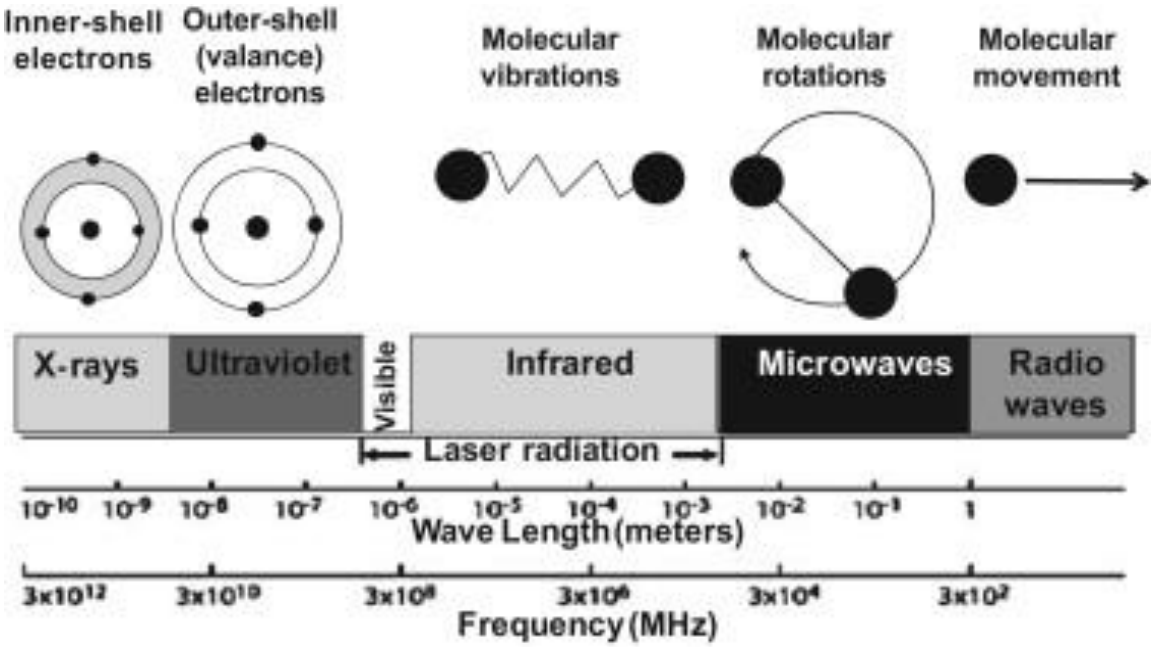


Figure 1. 3. Electromagnetic spectrum (Tyagi et al., 2013).

Microwave irradiation includes two mechanisms as thermal and athermal (Tyagi et al., 2013). Thermal effect states the heating caused by absorption of microwave energy and occurs as ionic polarization and dipole rotating (Tyagi et al.,2013 and Kostas et al., 2017). In presence of electric field, assymetric and polar molecules lean to rotate due to frequency to accommodate with the polarity of changing electric field. Friction between the rotating molecules expose to heat and increase in temperature (Uslu and Certel, 2006) and result in an internal temperature increase in materials (Figure 1.4.1). This mechanism refers to dipole rotating (Figure 1.4.2). Electric field also causes the movement of ions due to their charge and results with collision of ions. These collisions convert kinetic energy into thermal energy (Singh and Heldman, 1993).

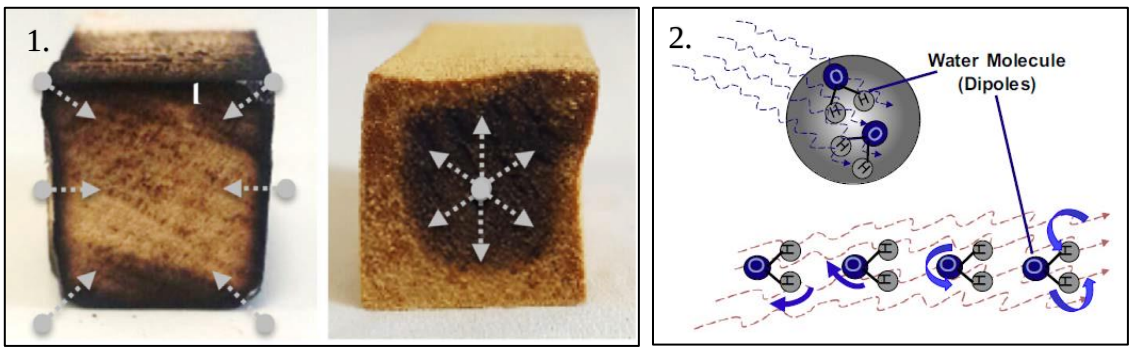


Figure 1. 4. 1. Temperature distributions and directions of heat transfer between conventional thermal treatment and microwave treatment (Kostas et al.,2017**) 2.** Dipole rotation (Tyagi et al., 2013).

Microwave energy have also athermal effects on sludge, other materials and solutions. This athermal effect includes the aligning of polar parts of macromolecules with poles of electric field and breaking hydrogen bonds (Tyagi et al., 2013).

Microwave treatment is an applicable method to treat waste sludge which consists dipolar molecules (Mawioo et al., 2016), so that its becoming one of the most popular sludge treatment methods in the literature. Microwave treatment is capable of eliminating pathogens in sludge, pyrolysis, sludge conditioning, stabilization and enhancing sludge solubilization by rupturing cell membrane as shown in Figure 1.5 (Gil et al., 2018 & Wu, 2008).

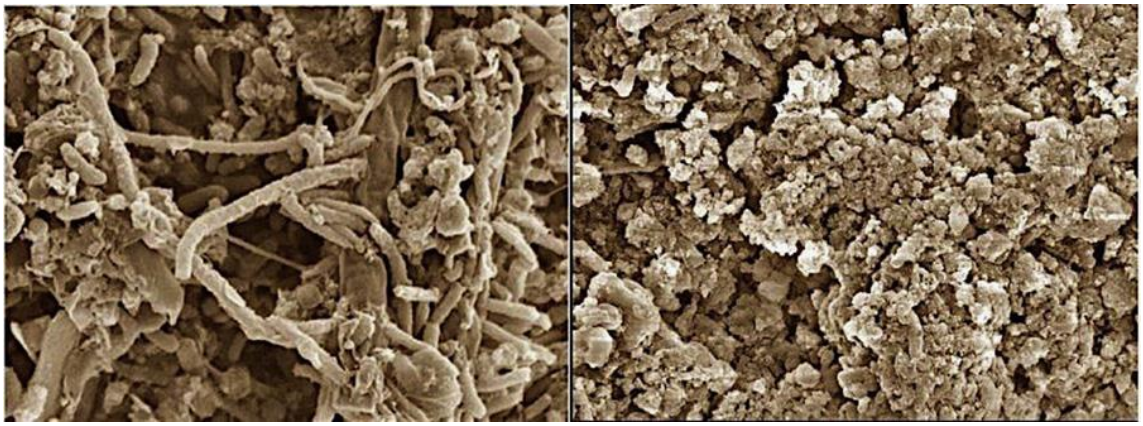


Figure 1. 5. Scanning electronic microscopic(SEM) photographs of sludge samples before and after MW treatment (Tyagi et al., 2013).

1.1.7. Ozone oxidation

Ozonation of sludge is one of the most effective methods for minimizing, disintegration and resource recovery. As a strong oxidant, ozone (O_3), can oxidize most organic and inorganic compounds. Ozone is also capable of destroying microorganisms and releasing intracellular compounds as a result of cell lysis. Ozone has three sequential effects on sludge; floc disintegration, solubilization and mineralization (oxidation of released intracellular organics to carbondioxide) (Chu et al., 2009). Inorganic elements like phosphorus, nitrogen and metals have also released during ozonation along with sludge solubilization (Sui et al., 2011).

Activated sludge is a mixture of microorganisms bound by extracellular polymeric substances, positive ions and granules (Zhang et al., 2009). Sludge granules are formed by adsorbing microorganisms onto small flocs; macromolecules like proteins, nuleic acids and organic acids generate a three-dimensional structure, and by bridging roles of

positive ions such as Mg^{2+} and Ca^{2+} porous zoogleas are formed in activated sludge. Ozone can destroy zoogleas and damage cell membrane and wall, as a result of which cell lysis occurs (Zhang et al., 2009).

Ozone is applied to the sludge for different purposes as solids reduction, digestibility of sludge, biomass reduction, enhancing settling properties, sludge dewatering and stabilization (Fall et al., 2018 & Vrantzky et al., 2005).

1.2. Application of Ozone in Sludge Treatment

Ozone was discovered by the C.F. Schonbein in 1839 (Morgan, 2009) and has used in municipal water treatment systems since 1907 in Nice and in 1910 in St. Petersburg (Guzel-Seydim et al., 2004). Ozone, as a strong oxidizing agent produced from molecular oxygen (O_2), is replacing chlorinated compounds for a variety of environmental applications including wastewater and polluted air treatment, and as a disinfectant (Park et al., 2006).

1.2.1. Ozone

Ozone is an allotropic form of oxygen (the existence of the same chemical substance in more than one form with different physical characteristics) with a chemical formula as O_3 and molecular weight 48g/mole. It is a pale blue, nearly colorless gas at room temperature with a pungent, characteristic odor (Srinath and Swaroopa, 2017). Major physical properties of pure ozone are given in Table 1.1. The schematic view of ozone molecular structure is shown Figure 1.6.

Table 1. 1. Major physical properties of pure ozone (O'Donnel et al., 2012).

Density	2.14 g/L
Boiling point	−111.9± 0.3°C
Melting point	−192.5± 0.4°C
Critical temperature	−12.1°C
Critical pressure	54.6 atm
Oxidizing Potential	2.07 V

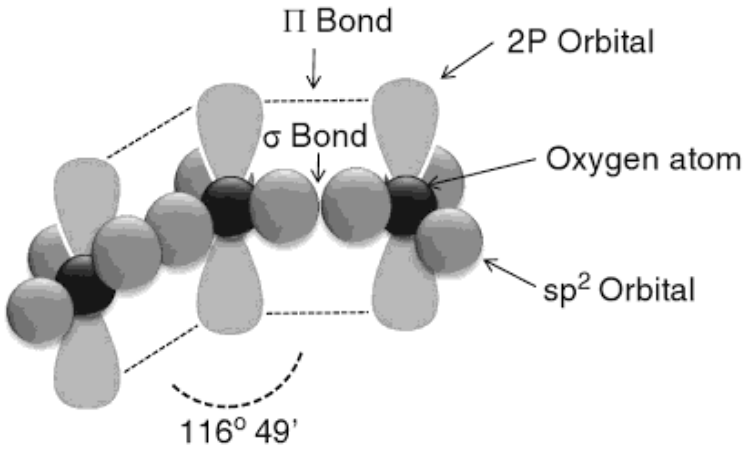


Figure 1. 6. The molecular structure of ozone (O'Donnel et al., 2012).

The ozone formation reaction is described in Equation 1.1 (Langais et al., 1991). Ozone is not a stable gas, decomposes rapidly and can not be accumulated without continuous generation (Guzel-Seydim et al., 2004), but still halflife of ozone is longer in gas form than aqueous (O'Donnel et al., 2012).

$3O_2 \leftrightarrow 2O_3$ ($\Delta H°$: + 284.5 kJ/mol $\Delta S°$: - 69.9 J/mol/degree) (Eq.1.1)

1.2.2. Ozone generation

Even though it decomposes slowly at ambient temperatures (in minutes) than higher temperatures (<1s), ozone is an unstable -thus highly oxidative- gas (Park et al., 2006 & Merizalde-Dobles, 2011). Therefore, it can not be stored and transferred, and needs to be generated on site to use. For generation of ozone, oxygen molecule should be seperated and the formed free radical oxygen should react with another oxygen molecule. However breaking O-O requires a huge amount of energy due to high bond energy as 498 kj/mol (West,1984 & Srinath and Swaroopa, 2017). Schematic diagram of an ozone generation system has shown in Figure 1.7.

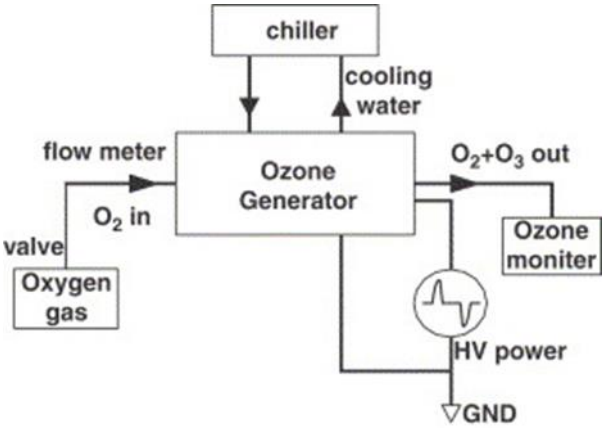


Figure 1. 7. Schematic diagram of a setup consisting of the ozone generator, a power supply, a cooling water system (chiller) for the ozone-generating space, an oxygen gas cylinder with flow meter and valve, and an ozone monitor to measure ozone output (Park et al., 2006).

There are several techniques for ozone generation, three most commonly used methods are corona discharge, electrolysis and UV radiation (Srinath and Swaroopa, 2017). Ozone is mostly generated by air and corona discharge (Wang et al. 2005). Corona discharge is occurred by passing of a high voltage through an air gap. This high voltage causes the breaking O-O bonds and allows forming O_3 molecules (Somalwar et al., 2014). Outline of a corona discharge generator is shown in Figure 1.8.

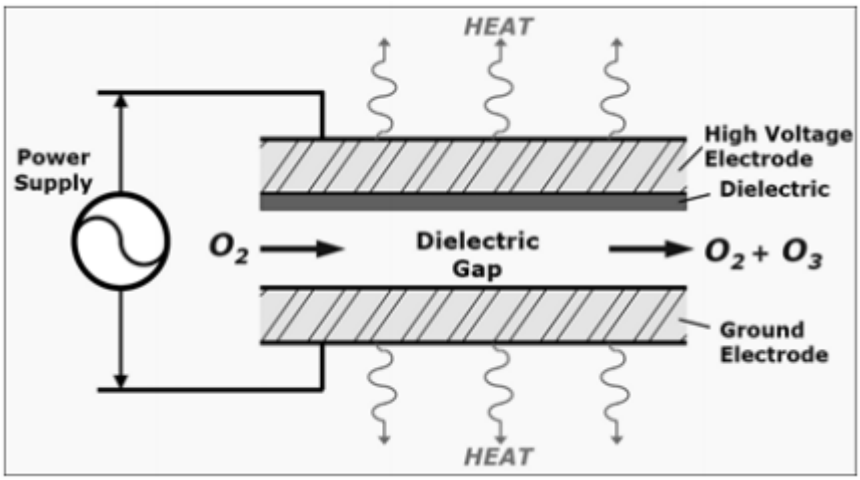


Figure 1. 8. Ozone generation by corona discharge process (Schiavon et al., 2012).

Electrolysis is an attractive alternative for ozone generation due to lower voltage need, hereby energy efficiency and eliminating side products like nitrous oxides (Wang et al., 2005). Ozone generation via water electrolysis has described in Equation 1.2 (Christensen, 2013). Besides water, different electrolytes like H_2SO_4 may be used for

this reaction too (Smith, 2011). Schematic view of electrolytic ozone generation design is shown in Figure 1.9.

$$3H_2O \rightarrow O_3 + 6H^+ + 6e^- \qquad \text{(Eq.1.2)}$$

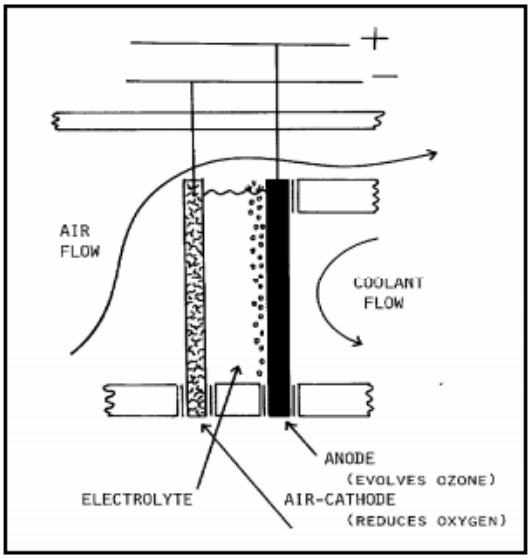


Figure 1. 9. Electrolytic ozone generation cell (Smith, 2011).

Charged particles can negatively affect the ozone generation efficiency. Thus, UV radiation is an alternative for ozone production (Eliasson & Kogelschatz, 1991). In nature, ozone is produced by sunlight and atmospheric oxygen. UV radiation (140-190nm) can supply the required energy of endothermic ozone production reaction (Srinath and Swaroopa, 2017). Oxygen molecules can be dissociated by absorbing light energy, and then oxygen can form ozone molecules by reacting with oxygen molecules. Mostly, 185nm optimized UV lamps are used for commercial ozone generation for small scale applications (Smith, 2011).

1.2.3. Behaviour of ozone in aqueous media

Ozone behaviour in aqueous solutions plays an important role in environmental applications (Ku et al., 1996). As mentioned before ozone is an unstable gas, and its stability is even lesser in aqueous form (O'Donnel et al., 2012). In pure aqueous solutions, ozone decomposes by multiple steps (Eriksson, 2005). This decomposition is explained by two mechanisms, Hoigné-Staehelin-Bader (1983) and Gordon-Tomiyasu-Fukutomi (1985). Chemical reactions involved in these mechanisms are shown in Table 1.2.

Table 1. 2. Decomposition of ozone in aqueous media (Eriksson, 2005).

Hoigné, Staehelin and Bader	Gordon, Tomiyasu and Fukutomi
$O_3 + OH^- \rightarrow HO_2 + O_2^-$	$O_3 + OH^- \rightarrow HO_2^- + O_2$
$HO_2 \leftrightarrow H^+ + O_2^-$	$HO_2^- + O_3 \rightarrow HO_2 + O_3^{\bullet -}$
$O_3 + O_2 \rightarrow O_3^{\bullet -} + O_2$	$HO_2 + OH \leftrightarrow H_2O + O_2^-$
$H^+ + O_3^- \leftrightarrow HO_3$	$O_3 + O_2 \rightarrow O_3^{\bullet -} + O_2$
$HO_3 \rightarrow HO^\bullet + O_2$	$H_2O + O_3^{\bullet -} \rightarrow OH + O2 + OH^-$
$HO^\bullet + O_3 \rightarrow HO_4$	$HO^\bullet + O_3^{\bullet -} \rightarrow O_2^- + HO_2$
$HO_4 \rightarrow HO_2 + O_2$	$HO^\bullet + O_3^{\bullet -} \rightarrow O_3 + OH^-$
$HO_4 + HO_4 \rightarrow H_2O_2 + 2\ O_3$	$HO^\bullet + O_3 \rightarrow HO_2 + O_2$
$HO_4 + HO_3 \rightarrow H_2O_2 + O_3 + O_2$	

In recent years, some revisions are added to ozone decomposition mechanism as shown in Table 1.3.

Table 1. 3. Revised decomposition of ozone in aqueous media (Gardoni et al., 2012).

$O_3 + OH^- \rightarrow HO_2^- + O_2$	$O_3 + OH^- \rightarrow HO_4^-$
$HO_2^- + O_3 \rightarrow HO_2^\bullet + O_3^{\bullet -}$	$HO_4^- \leftrightarrow HO^{\bullet 2} + O^{\bullet -}$
$HO_2^\bullet \leftrightarrow O^{\bullet -}{}_2 + H^+$	$HO_2^\bullet + O_2^{\bullet -} \rightarrow HO_2^- + O_2$
$O_3 + O_2^{\bullet -} \rightarrow O_3^{\bullet -} + O_2$	$O_3 + HO_2^- \rightarrow HO_5^-$
$O_3^{\bullet -} + H^+ \leftrightarrow HO^{\bullet 3}$	$HO_5^- \rightarrow 2O_2 + OH^-$
$HO_3^\bullet \rightarrow OH^\bullet + O_2$	
$O_3^{\bullet -} + OH^\bullet \rightarrow O_3 + OH^-$	

Ozone can react in aqueous solutions with two different ways, direct and indirect. While direct ozone reactions occur between ozone molecules and other compounds, indirect reactions involve hydroxyl radicals which produced by ozone decomposition (Gardoni et al., 2012). Stability of ozone in aqueous solutions are affected by some factors such as presence of organic and inorganic compounds, pH, ozone concentration and UV radiation (Gardoni et al., 2012). Organic materials in solution can react with ozone and hydroxyl radicals (Gunten et al., 2003). Decomposition of ozone starts with reacting with hydroxyl radicals. Thus, stability of ozone depends strongly on pH and alkalinity (Eriksson, 2005). High pH levels provide a remarkable concentration of OH^-, and accerelates the decomposition of ozone. After a series of experimental studies, Ku et al. (1996) showed that the rate of decomposition of ozone increases with increasing pH.

Also, pH levels less than 4 can change the initial mechanism of ozone decaying (Eriksson, 2005).

1.2.4. Effetcts of ozone on microorganisms

Bacteria consists of a cell wall surrounded by exo-polysaccharides, a cell membrane, and the cytoplasm containing the genetic information-carrying chromosome with an almost neutral pH (Langais et al., 1991). The cytoplasmic cell membrane may be a suitable site for ozone reaction due to its protein-rich content. Bacterial cell membrane contains lipids, proteins and C=C double bonds. Ozone react with -C=C- functional groups of unsaturated lipids, decomposition of cellular membrane and the release of cellular components, including extracellular polymeric substances (Chacana et al., 2017). When ozone penetrates the cell, the cytoplasm and the chromosome become preferred sites, since the nucleic acids are quickly degraded by ozone (Vranitzky and Lahnsteiner, 2000). Ozone damages nucleic acids by breaking aromatic structure and disrupts carbon-nitrogen bonds of proteins (Alexander et al., 2016). Cell lysis by ozone is described in Figure 1.10 visually.

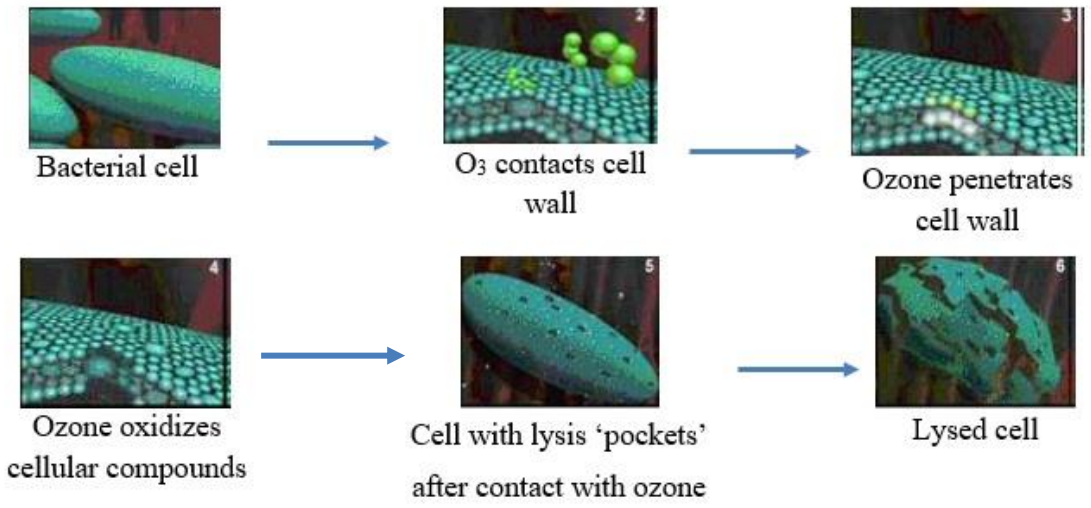


Figure 1. 10. Cell lysis by ozonation (Praxair,2008)

1.2.5. Sludge Ozonation

Sludge production causes some major issues in wastewater treatment plants due to high cost and difficulties of its removal (Semblante et al., 2017). Up to 65% of total cost of wastewater treatment plant is associated with excess sludge handling (Liu, 2003). Different physical and chemical methods have been studied for sludge disintegration

like ultrasound, thermal hydrolysis, mechanical disintegration and ozonation (Zhang et al., 2017). Among these methods, ozonation has been studied extensively as a promising method for consistent and reliable sludge reduction with an ability to reduce 50–100% of the excess sludge without toxic by-products (Fabiyi et al., 2007 and Zhang et al., 2017). Ozonation also improves the settling properties of sludge (Fall et al., 2018).

Ozone has been used for sludge treatment since mid-1990s and has been applied in full scale treatment plants successfully (Wang et., 2017). Ozonation can be applied for different purposes such as sludge minimization, resource recovery and enhancing anaerobic digestion efficiency as a pre-treatment (Figure 1.11.).

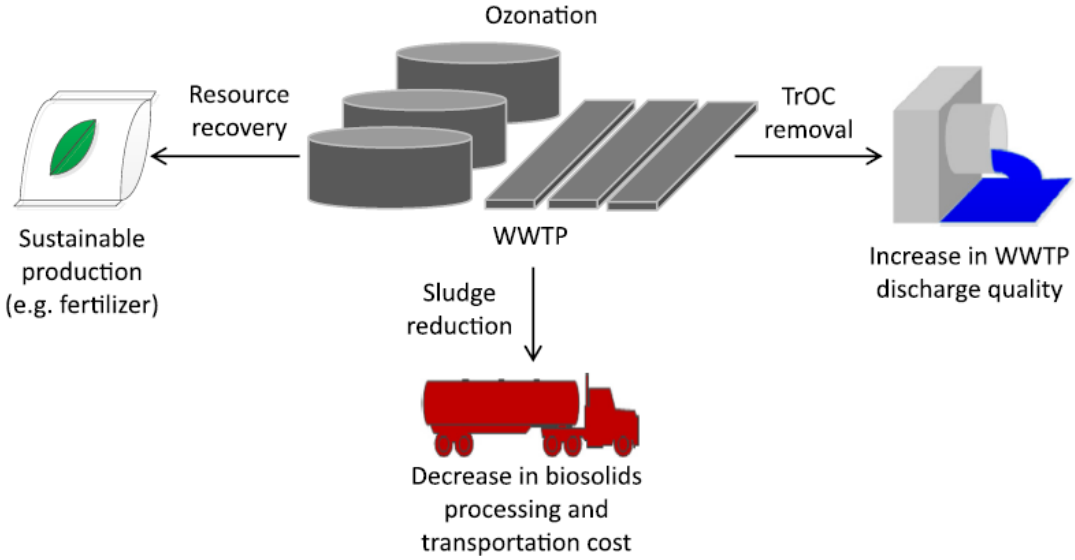


Figure 1. 11. Sludge management applications by ozonation (Semblante et al., 2017).

There are numerous studies in the literature which reported ozonation as an efficient pre-treatment for biogas production. While ozonation is not remarkably effective on primary sludge disintegreation (Chacana et al., 2017), it is capable of destroying microbial cell components and extracellular cell components, solubilizing macromolecules and predisposing refractory organic materials for biodegradation in activated sludge (Tian et al., 2015). Chacana et al. (2017) studied ozonation prior to anaerobic digestion and obtained 52% increase in methane yield with a 95% increase in methane production rate. In the same study, a temporary reduction in biomass activiy was also observed before the enhanced methane production. Bakhshi et al. (2017)

compared conventional anaerobic digestion (35º) of raw sludge and anaerobic digestion with low mesophilic temperature (20º) of ozonated sludge and obtained 35% more energy at 20 °C with ozonated than at 35 °C with raw sludge. Thus, sludge ozonation is a promising method for also enery savings in methane production.

Sludge minimization by ozonation is mainly driven by the floc disintegration (Figure 1.12.), solubilization and mineralization (Song et al., 2003 and Fall et al., 2018). Improvement of sludge settling and reduction of bulking by ozonation are well documented subjects in the literature. At the same time, ozone causes low filterability and dewaterability by releasing proteins into the soluble phase (Chu et al., 2009). Up to 100% reduction of sludge yield is posibble by ozonation (Semblante et al., 2017).

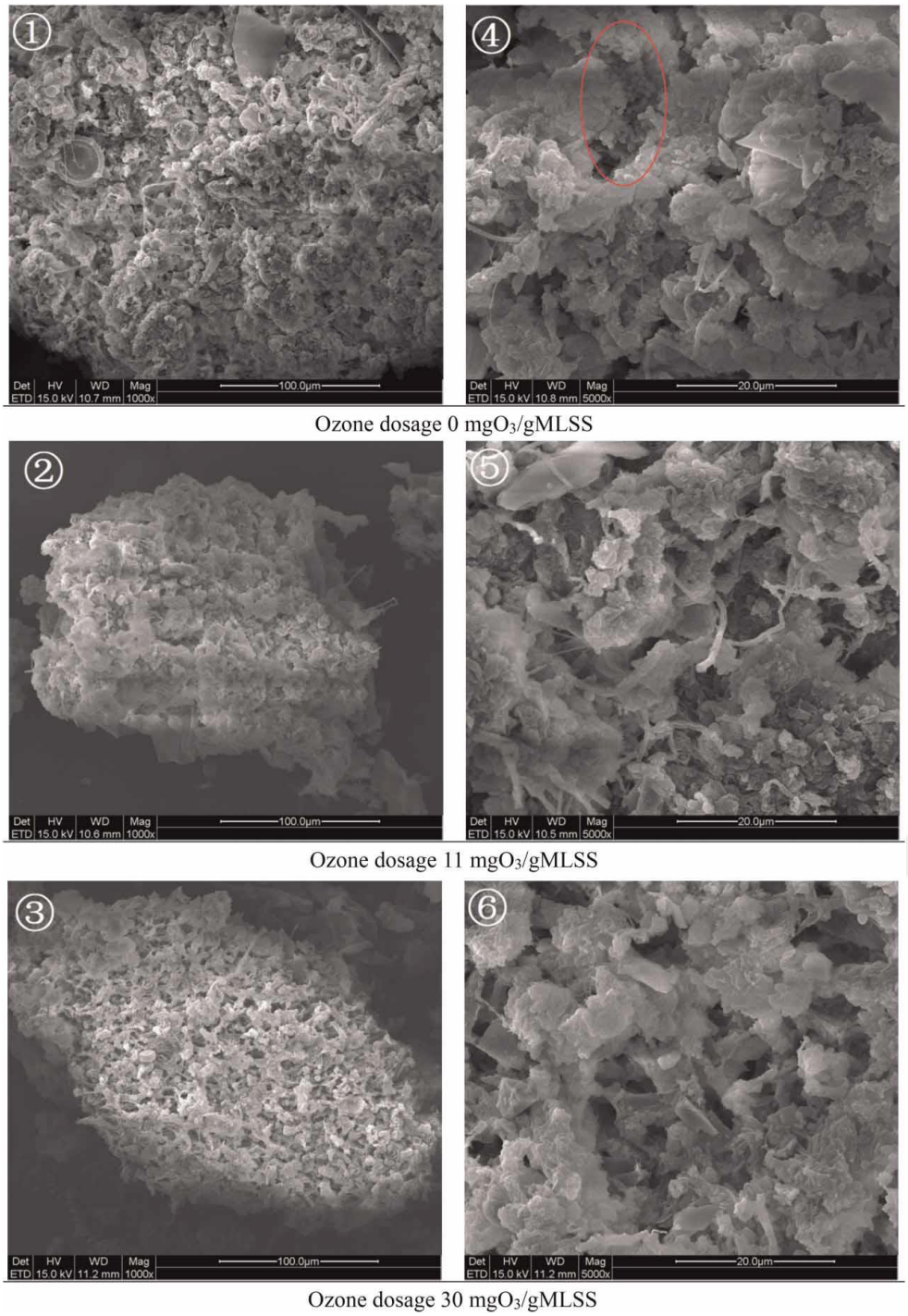


Figure 1. 12. 1. The surface morphology of raw sludge(x5000), **2.** The surface morphology of raw sludge (×1000), **3.** The surface morphology of ozonated sludge with 11mgO_3/gMLSS dosage (x5000), **4.** The surface morphology of ozonated sludge with 11mgO_3/gMLSS dosage (x1000), **5.** The surface morphology of ozonated sludge with 30 mgO_3/gMLSS dosage (x5000), **6. .** The surface morphology of ozonated sludge with 30mgO_3/gMLSS(x1000)(((Meng et al.,2015).

Another important result and profit of sludge ozonation is nutrient release and recovery as a result of sludge solubilization. Qiang et al. (2015) obtained 88% of TP and 85% of TN release during ozonation. Coupling ozonation and nutrient recovery systems has a

synergistic effect on operations of wastewater treatment plants. By reducing the phosphorus and nitrogen loads of plants, it prevents a possible decrease in effluent quality and also recovers the valuable sources as an applicable product (Semblante et al., 2017).

The ozonation also leads to some other changes in sludge characteristics. VSS/TSS ratio in sludge decreases by ozonation due to sludge solubilization and mineralization. The ratio of VSS/TSS was 78% in raw sludge and 73% after ozonation in the study of Bougrier et al. (2006). A decrease from 65% to 46% in the VSS/TSS ratio was reported by Chu et al. (2008) with pH decrease from 8 to 7.2. Higher pH drops by sludge ozonation may be found in the literature, Demir and Filibeli (2012) reported a decrease from 7.27 to 5.58. Since bound water content and particle size distribution is reduced by ozonation, zeta-potential shows an increasing trend (Park et al.,2003).

1.3. Phosphorus Recovery by Sludge Ozonation

1.3.1. Phosphorus

Phosphorus is a nonmetallic element with atomic number 15 which is discovered by Hennig Brandt in 1669 (Babrauskas, 2017). As a mineral, phosphorus is generally found in nature in its maximally oxidized state. Elemental phosphorus mostly exists in two major forms, white phosphorus and red phosphorus, but other allotropes like black phosphorus, diphosphorus, scarlet phosphorus can be found in nature. Some chemical properties of white phosphorus, which is the most abundant and important form, are shown in Table 1.4.

Table 1. 4. Characteristics of white phosphorus (Yon et al., 1983).

Atomic Number:	15
Symbol:	P
Atomic Weight:	30.973762
Density (g/cm^3):	1.828
Critical Temperature (ºC):	695
Critical pressute (atm):	82.8
Boiling Point (ºC):	280.5
Melting Point (ºC):	44.1
Heat Capacity (@25°C, cal/mol/degree):	22.73

Phosphorus is an essential and nonreplaceable element for all living organisms due to its essential role in nucleic acids and cellular metabolism functions (Borgestedt and Svanang,2011). Functions of phosphorus in organisms include energy storage, energy transfer (ATP&ADP) and being an integral part deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (Caldecott, 2009). Correspondingly, phosphorus is an important factor for growth of plants and so agriculture. Cooper et al. (2011) propose that, 90% of phosphorus use is for agriculture as feed or fertilizer. Phosphorus recources have accumulated over many millions of years, so it can be considered as a non-renewable source (Reijnders, 2014). There are estimations for the global reserve life of phosphorus between 25 and 160 years (Forrest et al., 2008). More optimistic views on existence time of phosphorus can be found literature, as far as 33rd century (Koppelaar and Weikard, 2013), however they all still have an expiration date for phosphorus sources. Therefore, lots of interest have been focused on finding new resources for phosphorus and activated sludge is a promising alternative due to its phosphorus-rich content.

1.3.2. Phosphorus in excess sludge

Wastewater and consequently activated sludge are nutrient rich pollutants. Around 90% of influent phosphorus and 100% of removed phosphorus, in case of biological wastewater treatment, accumulates in the activated sludge (Petzet et al., 2012).

Several technologies have been developed for phosphorus recovery from activated sludge, sludge ash and directly from liquid phase (Blöcher et al.,2012). Phosphorus recovery from sludge and sludge ash need a pre-treatment for disintegration at first.

With strong oxidizability, ozone can destroy flocs and microorganisms, release some intracellular and extracellular components including phosphorus. Release of phosphorus is indicated by an increase in orthophosphate and total soluble phosphorus concentrations, which includes acid hydrolyzable and organic phosphorus (Saktaywin et al., 2005). Qiang et al. (2015) obtained an increase from 3mg/L to 46.2mg/L in soluble TP concentration and 88% of released phosphorus was in the form of orthophosphate. The ratio of released orthophosphate to released soluble TP was 68% in the study of Sui et al. (2011). These results have shown the suitability of sludge ozonation for phosphorus recovery. Released phosphorus can be recovered by adsorption and

crystallization which includes struvite (MAP) and apatite (Ca-P) precipitation (Semblante et al. 2017).

Conditions for struvite precipitation with high efficiency and purity is a concerned subject in the literature. One of the well-known factors of struvite precipitation is pH. Studies in the literature have shown that higher pH results with higher struvite precipitation (Moulessehoul et al., 2017). Therefore, near to neutral pH levels mostly preferred, higher than pH:9 levels result in $Mg(OH)_2$ (Su et al.,2014), calcium phosphate (Doyle and Parsons, 2002) and $MgKPO_4$ (Hao et al.,2013) precipitations. Higher pH also causes smaller struvite particle size (Rahman et al.,2014). Mg:P ratio is an another important parameter for struvite precipitation. According to molecular formula, equal molecular amounts of Mg, PO_4-P and NH_4-N as 1:1:1 are enough to form struvite particles. Forming of struvite particles depends on supersaturation (Ye et al., 2016) and higher Mg:P ratios are recommended as 1.4 or 1.8 (Bi et al., 2014). Another reason for higher Mg:P recommendations is possible bounding of part of Mg with other substances like sCOD (Bi et al.2014). Also, temperature is a directly effective parameter for supersaturation and precipitation of struvite.

2. MATERIALS AND METHODS

2.1. Sludge Source

The aim of this study is to examine the relationship between solubilization degree of sludge, and pH, ozone dosage, microwave treatment. Sludge samples were obtained from the aeration tank and recycle line of the Paşaköy Advanced Biological Wastewater Treatment Plant.

2.2. Experimental Set-up

2.2.1. Phosphorus fraction

For phosphorus fractionation in different pH levels, experiments were carried out according to Xu et al. (2015) and standards, measurements and testing (SMT) protocol (European Commission) which was proposed by Pardo et al. (2004). The decided pH levels (Figure 2.1 &3.3) were adjusted and sludge samples were shaken at 25ºC and 250 rpm for 1 hour (Figure 2.1). After incubation, sludge samples were centrifuged at 3000 rpm for 10 minutes, and liquid and residue phases were separated immediately. In the liquid phase, reactive phosphorus and total phosphorus were measured by aminoacid reagent and persulfate digestion methods. Non-reactive soluble phosphorus (organic and acid hydrolyzable) was determined by substracting reactive phosphorus from total soluble phosphorus.

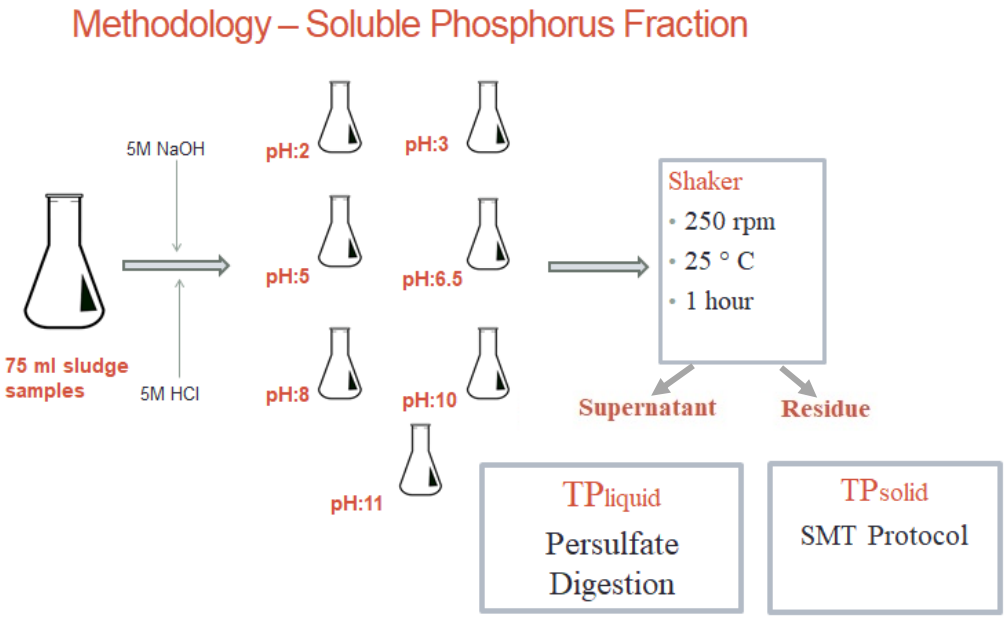


Figure 2. 1. Soluble phosphorus fraction methodology (Xu et al.,2015).

The residue of incubated sludge samples was dried for 24 hours at 105ºC and powdered by the help of mortar. Powdered samples were stored in a desiccator until examination. Non-apatite inorganic (P bound to Al, Fe, Mn oxides or hydroxides, NAIP), apatite inorganic (Ca-bound P, AP), organic P (OP), inorganic P (IP), total P (TP) fractionation experiments were run according to Figure 2.2. SMT protocol use NaOH to solubilize NAIP and HCl to dissolve AP. Total phosphorus concentration is determined by concentrated HCl.

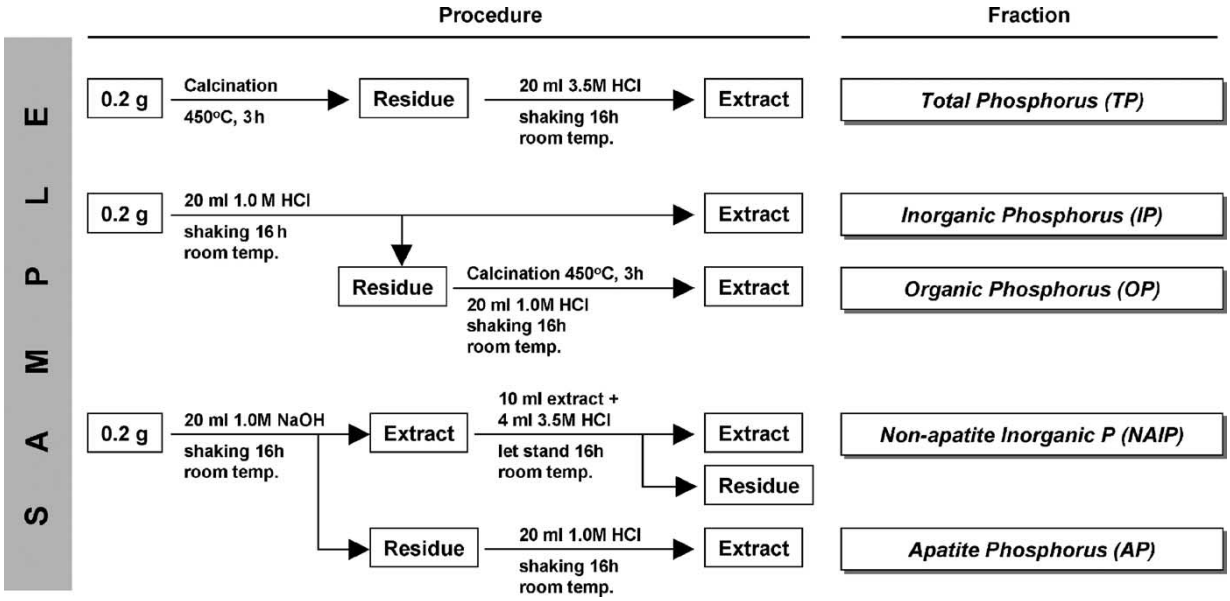


Figure 2. 2. SMT Protocol (Pardo et al., 2004)

2.2.2. Fluoresence in-situ hybridization (FISH)

For investigation of microbial species in sludge, and existence of phosphorus accumulating organisms (PAO) were determined by fluoresence in-situ hybridization. As the first step of FISH studies, sludge samples were washed with phosphate buffered saline and fixed by 4% paraformaldeydhe solution. Fixed cells have been applied to the slides, dehydrated in an ethanol series (50, 80, 100%) and then air- dried (46ºC). Slide were hybridized at 46ºC for 1.5 hours with hybridization buffer contains tris-HCl, NaCl, formamide, and sodium dodecyl sulphate and oligonucleotide probes. After hybridization, slides were washed with washing buffer (tris-HCl, NaCl, EDTA, and sodium dodecyl sulphate) for 20 minutes at 48ºC and dried. Hybridized slides were stained with Vectashield and monitored with Leica DM LB 2 Binocular Microscope

(Leica Microsystems, Germany). Oligonucleotide probes used in this study have shown in Table 2.1.

Table 2. 1. Oligonucleotide probes used in this study.

Probe	Sequence (5′–3′)	Target Site	Formamide (%)
EUB338	GCTGCCTCCCGTAGGAGT	16S	20
EUB338-II	GCAGCCACCCGTAGGTGT	16S	20
EUB338-III	GCTGCCACCCGTAGGTGT	16S	20
PAO651	CCCTCTGCCAAACTCCAG	16S	35
PAO846	GTTAGCTACGGCACTAAAAGG	16S	35
PAO462	CCGTCATCTACWCAGGGTATTAAC	16S	35

2.2.3. Sludge characterization

COD, MLSS, MLVSS, NH_4-N, PO_4-P, TP and metal concentrations of sludge samples were analyzed according to (Standard Methods, APHA, 20th edition). COD of mixed liquor (tCOD) and filtered by 0.45µm syringe filter (sCOD) sludge samples were examined according to Closed Reflux Colorimetric Method by using digestion solution (K_2Cr2O_7, H_2SO_4) and sulfuric acid reagent (Ag_2SO_4, H_2SO_4), and heating for 2 hours at a COD reactor preheated to 150ºC. After cooling, absorption of samples measured by a spectrophotometer at 600nm wavelength and concentrations obtained due to calculated equations by the calibration of potassium hydrogen phthalate standards. Sludge solubilizations were calculated according to Equation 2.1.

$$\text{COD release (\%)} = \frac{(sCOD_{after\ treatment} - sCOD_{before\ treatment})}{pCOD_{before\ treatment}} \text{x100} \qquad \text{(Eq.2.1)}$$

MLSS and MLVSS concentrations were calculated by drying and burning at 105ºC and 550ºC, respectively. Solid disintegration degree (%) have been determined by Equation 2.2. Ammonia concentrations were determined according to Nessler Method by using Mineral Stabilizer, Polyvinyl Alcohol and Nessler reagent. Absorbance of samples were measured at 425 nm. Ammonia values were given the ratio of the NH_4-N concentrations to the initial MLSS to normalize the values (mg NH_4-N/g$MLSS_{initial}$).

$$\text{Disintegration (\%)} = \frac{(MLSS_{after\ treatment} - MLSS_{before\ treatment})}{MLSS_{before\ treatment}} \text{x100} \qquad \text{(Eq.2.2)}$$

Aminoacid method was used for PO_4-P measurements. Ten minutes after the addition of molybdate and aminoacid reagents, absorbances were measured at 530 nm. Reactive phosphorus values have been given as the ratio to the initial MLSS (mg PO_4-N/g$MLSS_{initial}$). For total phosphorus, Persulfate Digestion Method was applied to the mixed liquor (TP) and filtered by 0.45µm syringe filter (soluble TP) sludge samples. After checking the pH of sludge by phenolphthalein 1 ml H_2SO_4 and 0.5 g $K_2S_2O_8$ were added and samples were heated in autoclave at 121ºC for 30 minutes. After digestion, aminoacid method is used for measurement of phosphorus. Non-reactive soluble phosphorus (organic phosphorus and acid hydrolyzable phosphorus) were calculated by substraction of reactive phosphorus from total soluble phosphorus.

Metal concentrations were determined by atomic absorption spectrophotometer (AAnalyst400, Perkin Elmer INC.USA). Cu, Fe lamps and Mg, Ca, Zn multilamps also obtained from Perkin Elmer. For intracellular metal concentrations EPA Method 3052 was applied by microwave and EasyPrep vessels. Experimental datas were analyzed by PASW Statistics 18 with Mann Whitney U test.

2.3. Phosphorus Recovery

To obtain phosphorus release due to sludge solubilization, a series of methods applied with different combinations. Ozonation and microwave treatment experiments were run with or without different pH pre-treatments. Ozonated sludge samples from each contact time were also treated with microwave. Schematic diagram of the procedure may be seen in Figure 2.3.

2.3.1. pH pre-treatment

To enhance ozonation and microwave efficiency, different pH pre-treatments were applied to the sludge samples. pH of sludge has adjusted as 2,4 and 10 by adding 1 N H_2SO_4 and 1 N NaOH. After pH adjustment, samples were mixed at 100 rpm for 30 minutes by mechanical strirrer and part of sludge have taken for standard measurements and microwave treatment.

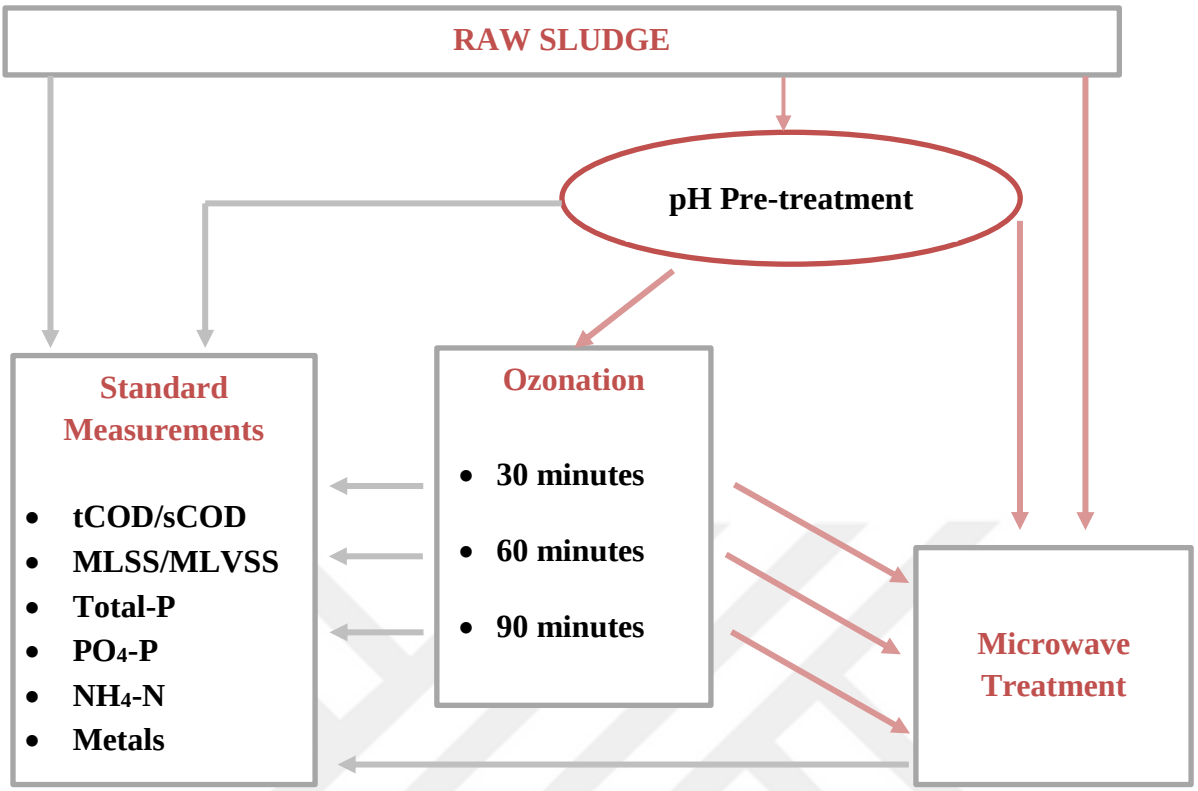


Figure 2. 3. Schematic diagram of the experimental procedure.

2.3.2. Sludge ozonation

Semi-batch system (liquid batch, gas continuous), which consisted of an ozone generator, ozon contactor and power supply was utilized to investigate the effect of ozone on sludge solubilization and phosphorus release. Ozone gas generated from pure oxygen with an Ozone Generator GL-1 (PCI Ozone &Control Systems INC., USA) (Figure 2.4). Raw or pre-treated sludge samples were ozonated in a cylindrical plexi contactor with an effective volume between 8 and 11 liters for 30, 60 and 90 minutes contact time. Detailed experimental conditions of sludge ozonation in batch tests and the sampling period are described in Table 2.2.

Table 2. 2. Experimental conditions of ozonation.

Date	Total Contact time(min)	Applied O_3 Percentage (%)	Air Flowrate (l/min)	Volume of Sludge(lt)	pH	Max. Dose Obtained ($mgO_3/gMLSS$)
14.04.2015	60	25	3.8	8	2	150
21.04.2015	60	25	2.8	10	2	99
05.05.2015	60	25	2.8	10	2	95
12.05.2015	60	25	2.8	10	2	89
24.06.2015	60	10	2.8	10	2	42
01.07.2015	60	25	2.8	8	2	122
06.07.2015	60	25	2.8	8	2	93
08.07.2015	60	25	2.8	8	2	109
14.07.2015	60	25	2.8	8	2	165
15.07.2015	60	25	2.8	8	2	117
20.07.2015	60	25	2.8	8	≈6.5	92
29.07.2015	60	25	2.8	8	2	109
09.12.2015	60	25	2.8	8	≈6.5	207
23.03.2016	60	25	2.8	8	≈6.5	274
05.04.2016	120	25	2.8	8	≈6.5	533
14.04.2016	90	25	2.8	11	≈6.5	242
21.04.2016	90	25	2.8	11	≈6.5	268
05.05.2016	90	25	2.8	11	≈6.5	156
20.07.2016	90	25	2.8	8	2	170
26.07.2016	90	25	2.8	10	4	316
02.08.2016	90	25	2.8	8	2	331
09.08.2016	90	25	2.8	8	4	457
15.08.2016	90	25	2.8	8	10	416
28.11.2016	90	25	2.8	8	10	376
11.05.2017	60	25	2.8	8	10	200
06.06.2017	90	25	2.8	8	2	332



Figure 2. 4. Ozone Generator GL-1.

Ozone gas was continuously bubbled into the contactor through a ceramic diffuser and sludge was mixed by overhead mechanical stirrer to provide homogenous condition during ozonation. Ozonated sludge samples were periodically withdrawn from the reactor and sample mixtures were divided into two parts. One portion of samples from each 30-minute period were treated by microwave and the other have measured for COD, PO_4-P, soluble TP, MLSS, MLVSS, NH_4-N and metals. Ozone dosage were determined according to Equation 2.3 and grouped as 50, 100, 150 etc. with high minus or plus values (as ± 25) due to varied dosages caused by varied MLSS concentrations.

$$\text{Ozone Dosage}(mgO_3/gMLSS) = \frac{787.5mg/min \times \text{Ozone \%} \times \text{Contact Time}(min)}{\text{Sludge Volume} \times \text{MLSS} \div 1000} \quad \text{(Eq.2.3)}$$

2.3.3. Microwave treatment

Samples from raw, pH pretreated and ozonated sludge were further treated with microwave. 50 ml sludge samples were taken into Xpress microwave vessels and treated with 1600W power and 95ºC temperature by Mars Xtraction (CEM Corporation, USA) which shown in Figure 2.5. Temperature profile of the procedure consisted 15 minutes increasing, 30 minutes holding and 15 minutes cooling phase.



Figure 2. 5. Mars Xtraction.

2.3.4. Struvite Precipitation

Struvite precipitation studies were carried out in two phases. Initially, batch precipitation experiments were run in a one liter beaker with pH:8.5, pH:9, pH:9.5 and pH:11 with Mg:N:P ratios between 1.4:1:1 and 1.8:1:1 (Bi et al.,2014). Precipitates were collected and dissolved in 2% HNO_3 solution. Mg and Ca concentrations of solutions were determined by AAnalyst400 and pH:8.5 was chosen for the rest of the experiments due to highest Mg and lowest Ca concentrations.

As shown in Figure 2.6, a semi-continuous lab-scale precipitation system was set up with sludge feed pump, pH dosing pump, Mg and NH_4-N dosing pumps(Seko), overhead mechanical stirrer and plexiglass reactor. The curved precipitation reactor was filled by sludge feed pump with supernatants of ozonated and microwave irradiated sludge and pH of the system was adjusted as 8.5 automatically by the pH dosing pump with 1M NaOH solution and monitored continuously by a pH sensor. Where necessary, Mg and NH_4-N dosing pumps were operated manually to obtain Mg:N:P ratio as 1.5:1:1 with 1M $Mg(Cl)_2$ and 1M NH_4Cl. Supernatant was mixed at 50 rpm for two days and allowed for two hours for settling the precipitates, and than new supernatant was given to the system via sludge feed pump. Precipitate samples were collected, air

dried and mortored for XRD analysis. XRD analyses were carried out by an another department by service procurement.



Figure 2. 6. Lab-scale struvite precipitation system.

3. RESULTS & DISCUSSION

3.1. Sludge Characteristics

In the scope of this study, 26 batch experiments were performed with different exposure times, ozone dosages and pretreatments to maximize the efficiency of ozonation on COD and P release from activated sludge. At the beginning of each batch experiment, sludge characterization tests were carried out. As proven by the standard deviations in Table 3.1, sludge characteristics were mostly inconsistent for both recycle line and aeration tank samples except the reactive phosphorus to suspended solid ratio while total phosphorus was around 2% of sludge by weight. Fluorescence In-Situ Hybridization (FISH) studies were also carried out to prove existence of phosphorus accumulating organisms (Figure 3.1).

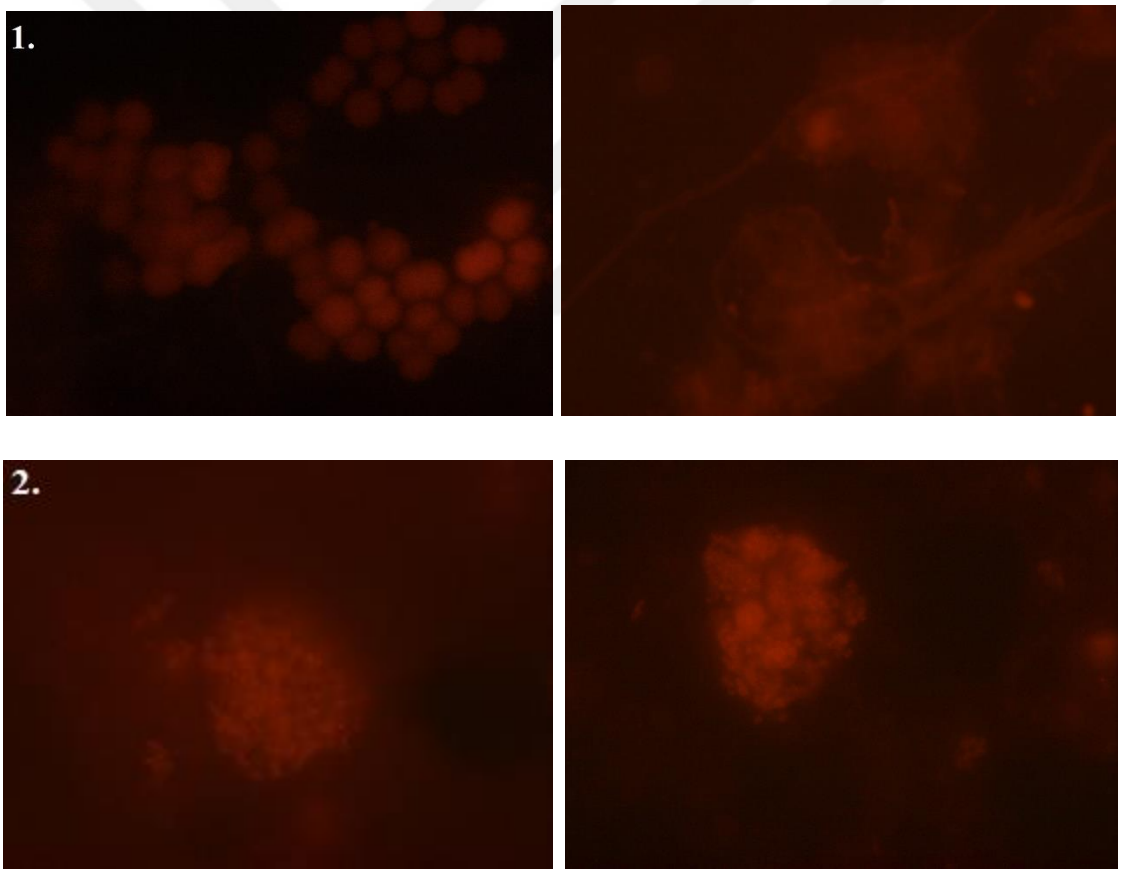


Figure 3. 1. 1. Identification of general bacteria in activated sludge by FISH analysis, **2.** Identification of phosphorus accumulating organisms in activated sludge by FISH analysis.

Table 3. 1. Characteristics of sludge samples from recycle line and aeration tank of Paşaköy Advanced Biological Wastewater Treatment Plant.

	Recycle Line			Aeration Tank		
	Average	Minimum Value	Maximum Value	Average	Minimum Value	Maximum Value
PO_4-P (mg/L)	29.9 (σ =15)	6	56.3	14 (σ =12)	2	38
TP (mg/L)	273	273	273	129 (σ =14)	117	144
PO_4-P /MLSS (mg/g)	1.93 (σ =1)	≈0	4	1.9 (σ =2)	≈0	5
NH_4-N (mg/L)	3 (σ = 2)	≈0	6.8	3 (σ =3)	≈0	9
sCOD (mg/L)	207 (σ=246)	≈0	938	110 (σ=120)	≈0	425
tCOD (mg/L)	11401 (σ =3689)	5000	17216	6979 (σ=2704)	3250	13625
MLSS (mg/L)	15797 (σ=2590)	10920	19520	7266 (σ=1166)	4460	8820
MLVSS (mg/L)	6602 (σ=1571)	3450	10800	4075 (σ=1114)	1900	5750
Mg (mg/L)	18 (σ =7)	13	28	20 (σ =6)	15	31
Ca (mg/L)	94 (σ =61)	25	175	75 (σ =25)	54	117

Consistent with the aim of this study, phosphorus content of the sludge has investigated in detail. Soluble and solid fractions of phosphorus have determined as shown in Figure 3.2 and 3.3. As similar to the results exposed by Xu et al. (2015), total soluble phosphorus (sTP) concentration has increased under both alkaline and acidic conditions and even more under acidic pH levels than alkaline. However, soluble phosphorus fractionation is differed from Xu et al. (2015). In our study, non-reactive soluble

phosphorus percentage were higher at acidic and neutral pH levels as15% and 40% than Xu et al. (2015) ≈4% and 10%. This discrepancy might be due to acid hydrolyzable phosphorus which was not taken into account in the study of in Xu et al. (2015) which all non-reactive soluble phosphorus was accepted as organic phosphorus. Despite, these results comform with the proposal of Xu et al. (2015) and Xie et al. (2011) about dissolution of inorganic-P and polyphosphate under acidic conditions in addition to release of organic phosphorus under alkaline condition.

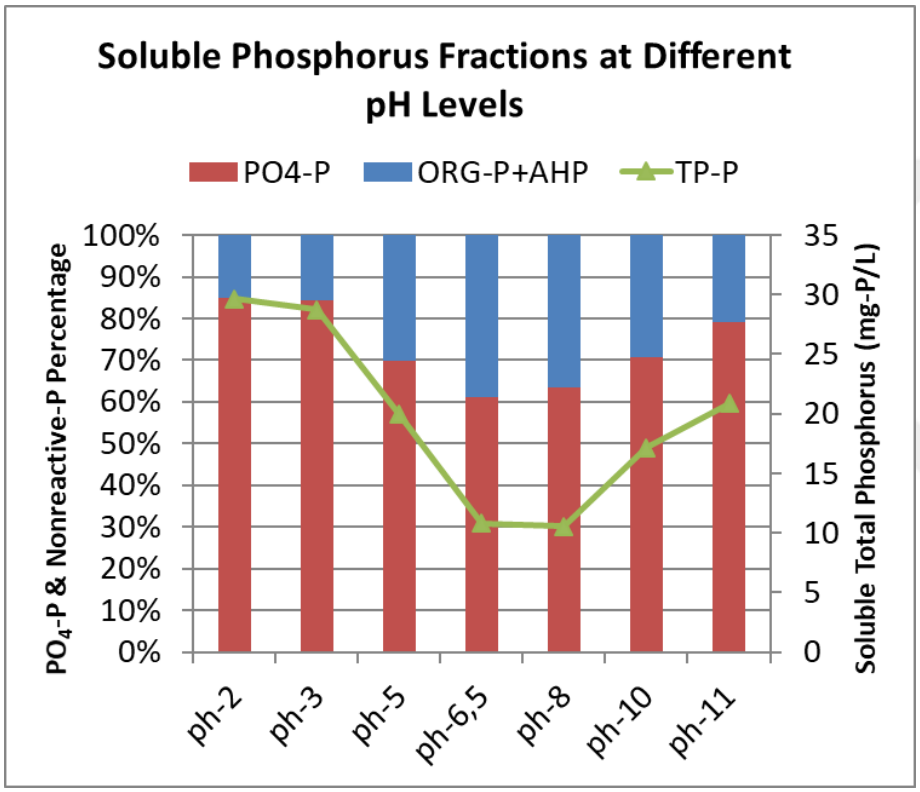


Figure 3. 2. Soluble phosphorus fractions under acidic and alkaline pH conditions.

Table 3. 2. Soluble phosphorus fractions under acidic and alkaline pH conditions.

	pH:2	**pH:3**	**pH:5**	**pH:6.5**	**pH:8**	**pH:10**	**pH:11**
Soluble Total-P (mg/L)	29.6	28.7	20	10.8	10.5	17.1	20.9
PO_4-P (mg/L)	25.2	24.3	14	6.6	7	12.1	16.5
ORG-P & AHP (mg/L)	4.4	4.5	6	4.2	3.9	5	4.4

As shown in Figure 3.3, different pH conditions also effect the solid fraction of phosphorus in activated sludge. Under alkaline conditions, organic phosphorus was released from sludge into the solution and apatite phosphorus content in sludge increased as a result of phosphorus precipitation. A slight decrease in non-apatite inorganic phosphorus was also observed under higher pH levels. These results conform with the argument of Ruban et al. (2001) as both inorganic and organic phosphorus are releasable under different pH levels and convenient for phosphorus recovery.

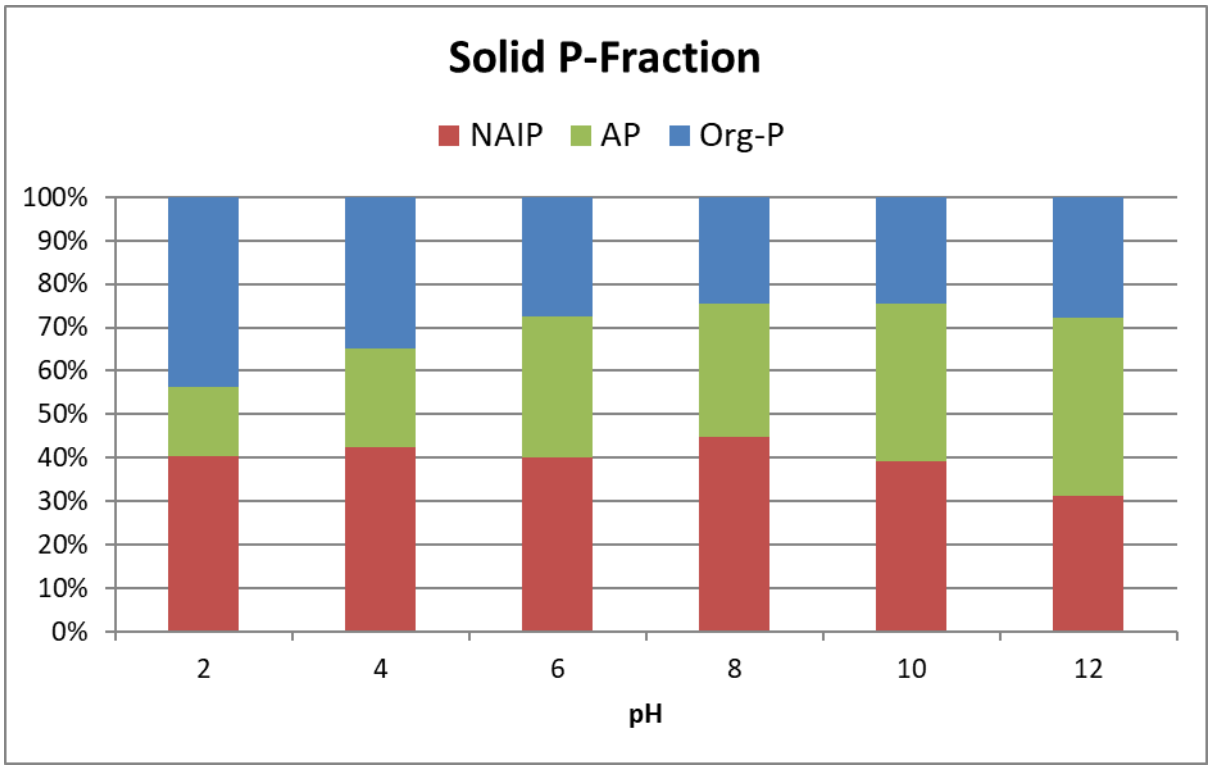


Figure 3. 3. Solid phosphorus fractions under different pH conditions [apatite (AP), nonapatite(NAIP) and organic phosphorus(OP)].

3.2. Sludge Disintegration

As known, ozone gas attacks to the cell membrane and causes release of the intracellular components into the solution. During all the batch experiments with different conditions, COD concentration in the supernatant increased with ozonation. Ozonation also caused mineralization of total organic matter, which is oxidation of organic matter into carbon dioxide gas.

3.2.1. Sludge disintegration with different pH treatments

Sludge solubilization degree in the experiments were determined by COD release, MLSS and MLVSS solubilization. As shown Figure 3.4, higher COD release was observed under acidic conditions than pH:10 as mentioned in Gao et al. (2011). Although Gao et al. (2011) proposed, in the same study, that pH:11 causes more sludge solubilization than acidic conditions, working at the proposed level of pH was impossible in this study due to excessive foaming problem. MLSS solubilization was also higher at acidic pre-treatment experiments probably due to solubilization of inorganic precipitates in addition to floc solubilization and cell lysis.

Table 3. 3. COD release, MLSS and MLVSS solubilizations after different pH treatments.

	COD Release (%)			**MLSS Solubilization (%)**			**MLVSS Solubilization (%)**		
	pH:2	**pH:4**	**pH:10**	**pH:2**	**pH:4**	**pH:10**	**pH:2**	**pH:4**	**pH:10**
Mean	2.4	2.3	1.3	8.0	7.0	3.8	5.8	6.0	5.3
Median	2.8	2.3	0.7	8.3	7.0	3.8	5.0	6.0	2.8
Maximum	4.6	3.7	3.2	15.1	7.7	4.9	11.2	9.7	11.7
Minimum	0.3	0.9	0.0	4.2	6.3	2.7	2.2	2.2	1.5

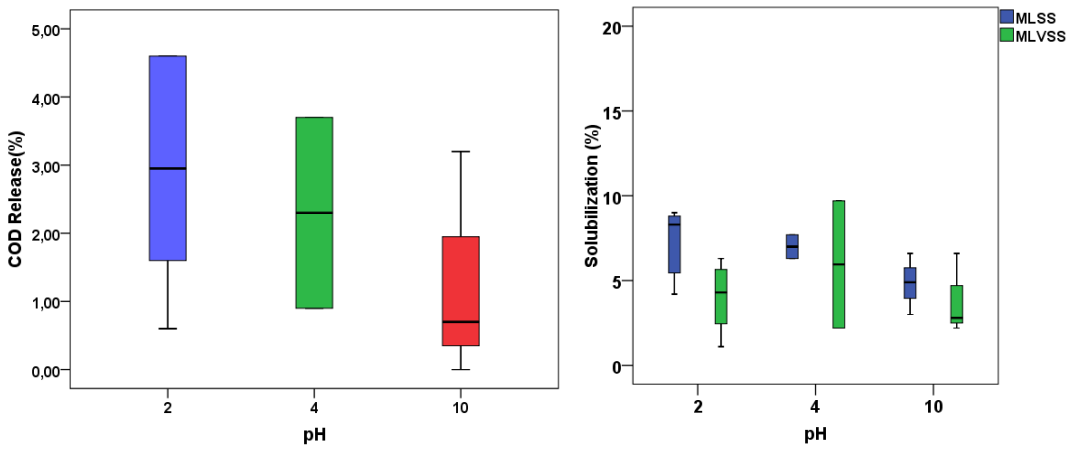


Figure 3. 4. COD release, MLSS and MLVSS solubilizations after different pH treatments.

3.2.2. Sludge disintegration with ozonation

As mentioned by Chu et al. (2009), sludge solubilization by ozonation consists different decomposition reactions as solids disintegration, cell lysis, and mineralization. 10-60% COD releases due to sludge solubilization at 10-160 mg O_3/gMLSS ozone dosages are reported in the literature (Semblante et al., 2017). It is claimed that, excessive ozone dosages resulted with the similar releases with lower dosages, due to radical scavengers (Fall et al., 2018). Naso et al. (2008) was observed same COD releases at 50 and 370 mg O_3/gMLSS ozone dosages. But in the study of Demir and Filibeli (2012), 56.19% COD release was observed due to 500 mg O_3/gMLSS ozonation. As shown in Figure 3.5 and Table 3.4, highest COD release obtained as 20.2 % at 250 mg O_3/gMLSS dosage in our study.

Table 3. 4. COD release during ozonation.

COD Release (%)				
	50 mgO_3/gMLSS	**100 mgO_3/gMLSS**	**150 mgO_3/gMLSS**	**250 mgO_3/gMLSS**
Mean	2.8	10.3	14.1	20.2
Median	2.3	10.7	13.2	21.3
Maximum	5.2	12.3	18.7	31.3
Minimum	1.3	7.5	11.5	7.2

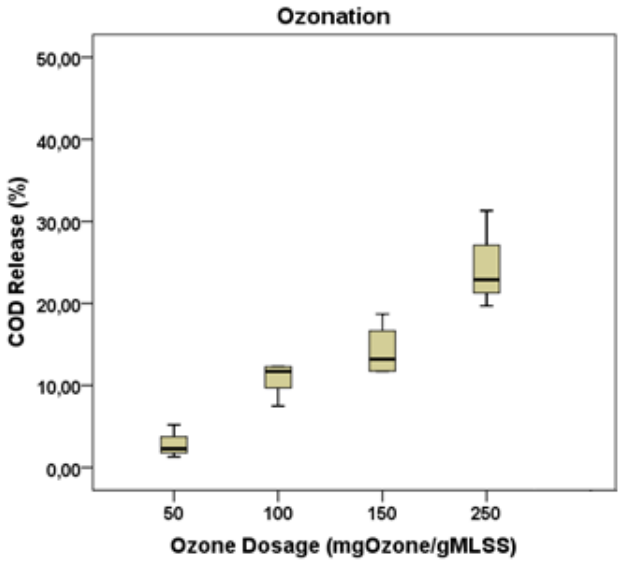


Figure 3. 5. COD release during ozonation.

On the other hand, high amount of soluble COD has disappeared due to mineralization, which is the reaction of ozone with sCOD and oxidation to CO_2. Mineralization is a prevalent phenomena in the literature. Zhang et al.(2009) reported that ozone dosages higher than 80 mgO_3/gDS cause mineralization. Chacana et al. (2017) predicted this limit as 157 mg O_3/gCOD. In the study of Chu et al. (2008) 16-21% mineralization was observed at 60-160 mgO_3/gMLSS ozone dosages. In our study, mineralization was observed even at low dosages and reached to 27.7% at 150 mgO_3/gMLSS as shown in Figure 3.6. Higher mineralization than literature might indicates a need for optimization of ozonation system to provide the contacting of ozone gas with the particulate sludge rather than soluble organics like placing diffusers at the bottom of an elongated reactor.

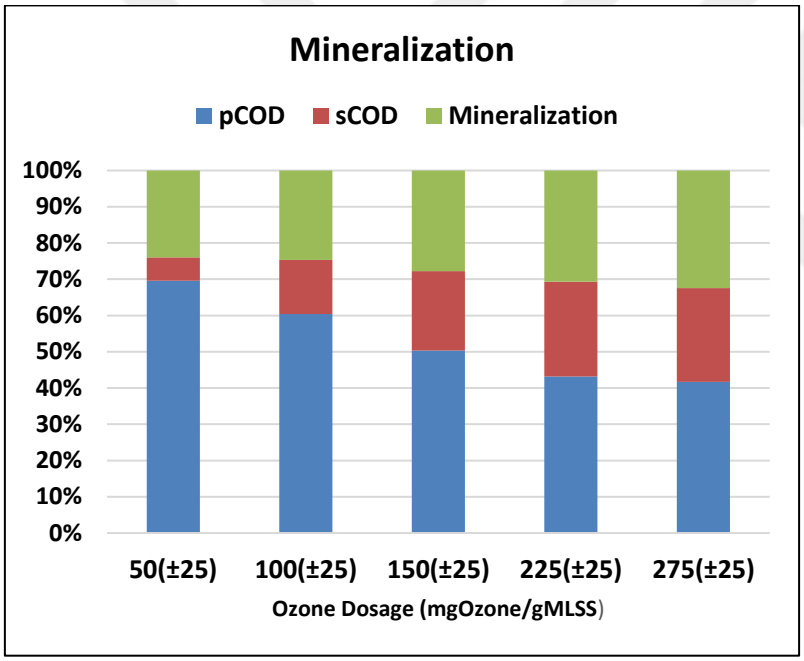


Figure 3. 6. Mineralization during ozonation.

MLSS and MLVSS are another markers for sludge solubilization. MLVSS solubilization occurs by breakdown of sludge flocs which are microbial aggregates bound together by extracellular polymeric substances (Semblante et al., 2017). Zhang et al. (2017) achieved 13.46% and 11.85% MLSS and MLVSS solubilizations at 50.4 mg O_3/gMLSS. In the study of Tian et al (2015), MLVSS solubilization was 21.1% at 48 mgO_3/gMLSS ozone dosages. Qiang et al. (2015) also observed the nearly the same values at same dosage. Demir and Filibeli (2012) obtained higher solid reduction with

77.78% MLSS and 71.59% MLVSS solubilization at 50 mg O_3/gMLSS. In this study, 23.2% MLSS and 26.1% MLVSS solubilization were observed at 100 mgO_3/gMLSS ozonation (Figure 3.7& Table 3.5). Higher ozone dosages resulted with greater solubilization efficiencies and 26.8% MLSS and 33.1% MLVSS solubilization were achieved at 250 mgO_3/gMLSS ozone. In contrast to literature data, MLVSS solubilization was slightly higher than MLSS. It might be due to the presence of inorganic particulates on which ozonation has no effect.. Zhang et al. (2017) reported that only one part of inorganic sludge disintegrated until 37.8 mgO_3/gMLSS ozone dosage and sludge disintegration stayed almost constant at higher dosages. Another possible reason of lower MLSS solubilization might be adsorption of released intracellular inorganics like heavy metals on sludge particles as mentioned by Zhang et al. (2017).

Table 3. 5. MLSS and MLVSS solubilization at different ozone dosages.

MLSS Solubilization (%)	**Ozone Dosage (mgO_3/gMLSS)**	**100**	**150**	**250**
	Mean	23.2	23.4	26.8
	Median	23.3	23.4	30.1
	Maximum	38.8	31.4	42.2
	Minimum	7.4	15.4	10.4
MLVSS Solubilization (%)	**Ozone Dosage (mgO_3/gMLSS)**	**100**	**150**	**250**
	Mean	26.1	27.9	33.1
	Median	26.1	27.9	33.1
	Maximum	40.7	34.0	43.1
	Minimum	11.7	21.9	19.7

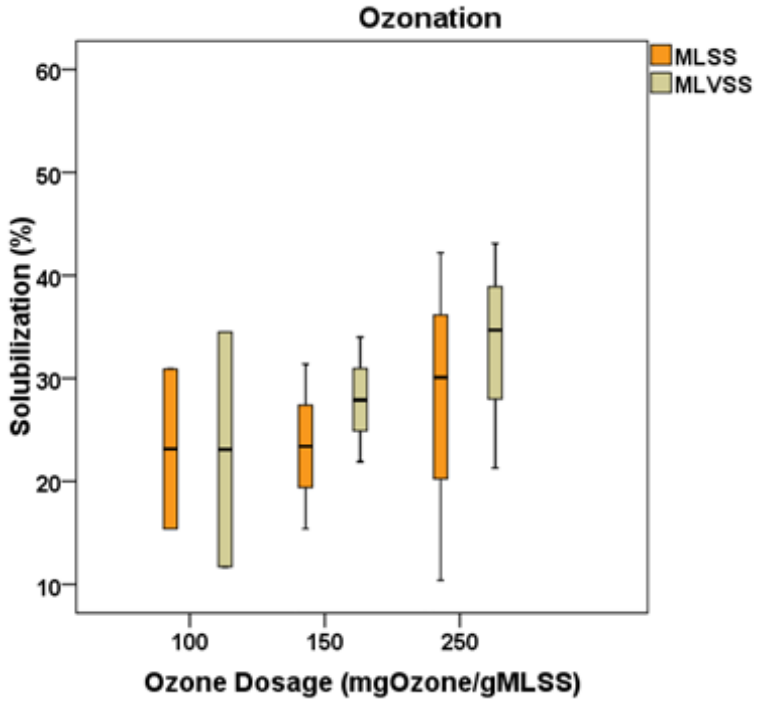


Figure 3. 7. MLSS and MLVSS solubilization at different ozone dosages.

3.2.3. Sludge disintegration with ozonation & different pH pre-treatments

pH pre-treatments prior to ozonation enhanced the sludge solubilization at low ozone dosages in accordance with the proposal of Subha and Muthukumar (2012). In terms of COD release, sludge solubilization have increased from 2.8% to 5.2% at 50 mg O_3/gMLSS dosage by pH:2 pre-treatment. As ozonation continues, the difference between neutral and acid pre-treated sludge solubilizations decreases gradually. At higher ozone dosages, solubilization in neutral sludge was higher than acid pre-treated sludge. At 250 mgO_3/gMLSS, COD release were 20.2% for neutral sludge and 10% for pH:2 pre-treated sludge at 225 mgO_3/gMLSS.

In acid pre-treatment experiments, effects of pH:4 on ozonation also investigated. As shown in Figure 3.8 and Table 3.6, COD release was 5.3% at 100 mg O_3/gMLSS and 18.7% at 300 mg O_3/gMLSS. Once again, the results showed the enhancing effects of acidic pre-treatments at low ozone dosages and reverse effects on higher dosages. pH:2 pre-treatment becomes an advantage at low ozone dosages, however acidic condition becomes a disadvantage at higher ozone dosages. It might be the result of the blocking of forming hydroxyl radicals by higher H^+ concentrations at low pH levels. On the other hand, there were no significant differences between COD releases from recycle line and

aeration tank sludges (p:0.325 for ozonation with pH:2 pre-treatment experiments). COD releases during ozonation with pH:2 and pH:4 pre-treatments are shown in Figure 3.8 and Table 3.6.

Table 3. 6. COD release during ozonation with pH:2 and pH:4 pre-treatments.

COD Release (%)					
pH:2	**Ozone Dosage (mgO_3/gMLSS)**	**50**	**100**	**225**	**325**
	Mean	5.2	7.2	10.0	11.4
	Median	4.6	6.9	10.0	11.4
	Maximum	14.9	22.7	12.2	13.1
	Minimum	1.8	1.2	7.8	9.7
pH:4	**Ozone Dosage (mgO_3/gMLSS)**	**100**	**175**	**300**	
	Mean	5.3	10.9	18.7	
	Median	5.3	10.9	18.7	
	Maximum	5.3	16.5	26.5	
	Minimum	5.3	5.3	10.9	

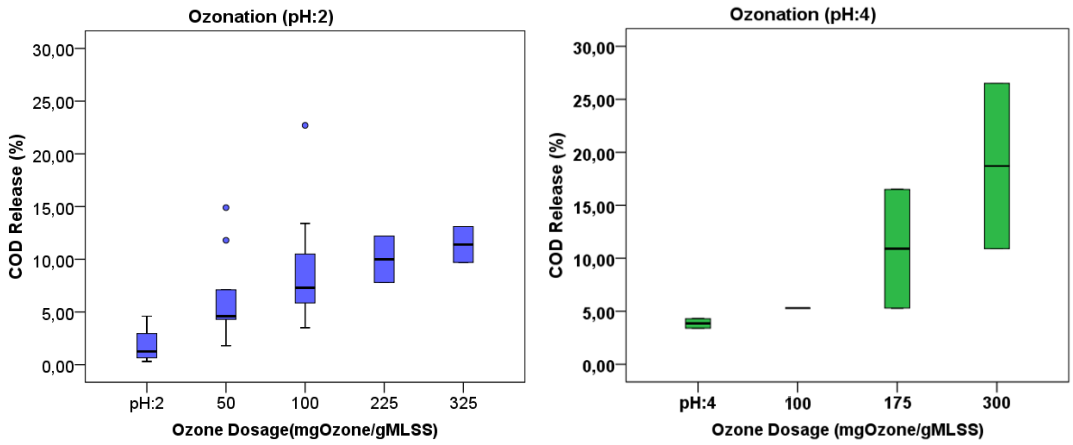


Figure 3. 8. COD releases during ozonation with pH:2 and pH:4 pre-treatments.

MLSS and MLVSS solubilizations were also influenced by acidic pre-treatments. Unlike the cell lysis effect of low pH levels, MLSS and MLVSS solubilizations were decreased by acidic pre-treatment as shown in Figure 3.9 and Table 3.7. While 26.5% MLSS and 33.1% MLVSS solubilization could be obtained by ozonation at 250 mg

O_3/gMLSS without any pre-treatment, MLSS and MLVSS solubilizations achieved by ozonation were 20.7% and 20.3% at 325 mg O_3/gMLSS with pH:2 pre-treatment. In contrast to ozonation at neutral pH, MLSS solubilization was higher than the MLVSS solubilization at 100 mg O_3/gMLSS. It might indicate the dissolution of some inorganics by acidic conditions in addition to cell lysis and breakdown of sludge flocs.

Table 3. 7. MLSS and MLVSS solubilization with ozonation & acid pre-treatment.

MLSS Solubilization (%)		**pH:2**			**pH:4**	
	Ozone Dosage (mgO_3/gMLSS)	**100**	**225**	**325**	**175**	**300**
	Mean	14.2	13.8	20.7	26.8	32.0
	Median	16.1	13.8	20.7	26.8	32.0
	Maximum	18.5	18.2	22.9	29.1	36.2
	Minimum	6.5	9.4	18.6	21.8	27.7
MLVSS Solubilization (%)		**pH:2**			**pH:4**	
	Ozone Dosage (mgO_3/gMLSS)	**100**	**225**	**325**	**175**	**300**
	Mean	12.6	19.2	20.3	26.8	34.1
	Median	12.8	19.2	20.3	26.8	34.1
	Maximum	17.1	24.9	25.4	33.3	39.5
	Minimum	7.3	13.6	15.2	20.3	28.8

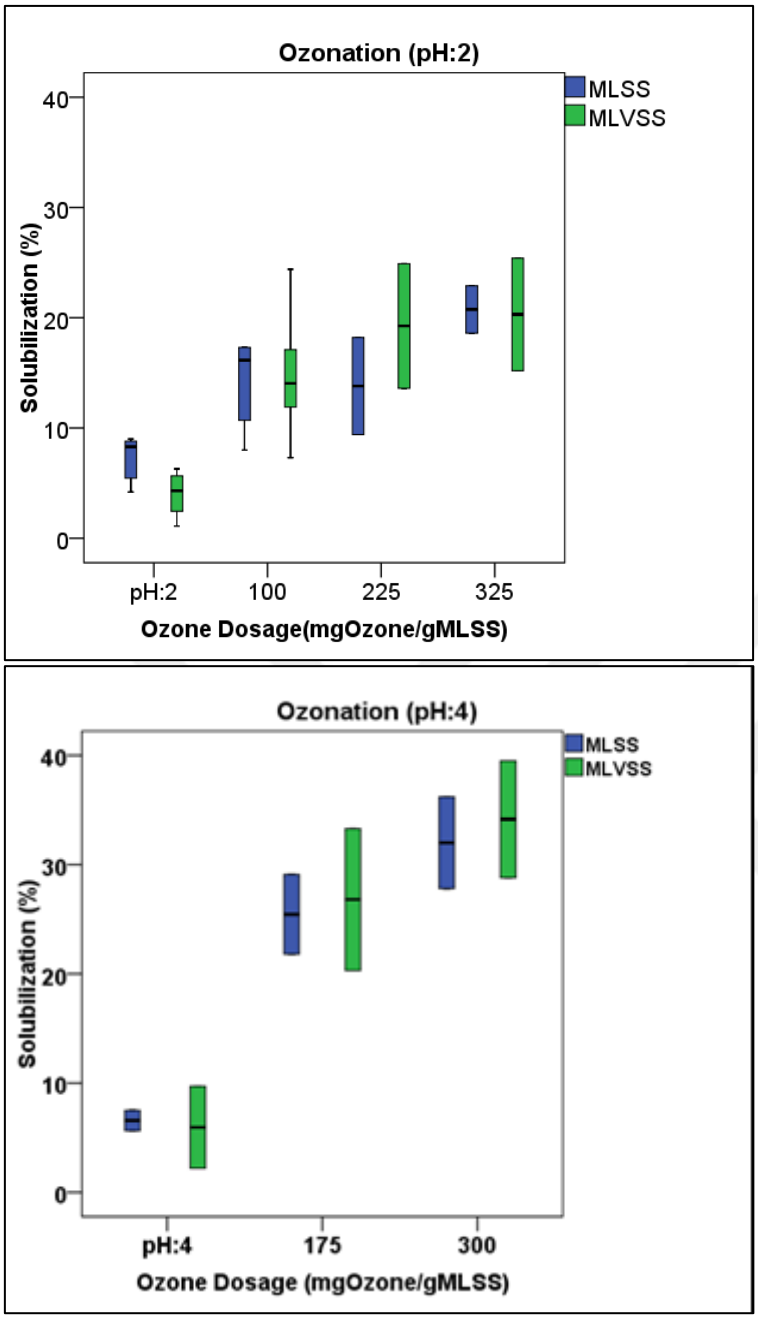


Figure 3. 9. MLSS and MLVSS solubilizations during ozonation with pH:2 and pH:4 pre-treatments.

Alkaline pre-treatment might be another alternative for enhancing ozonation efficiency by forming hydroxyl radicals. Higher COD release takes place under alkaline conditions as proven by Maspolim et al. (2015) and Gao et al. (2011). It shows that alkaline pre-treatment causes decomposition of acidic groups in EPS and repulsions between the negatively charged EPS, enhances cell lysis more than acidic pre-treatment (Chen et al., 2007). Kumar et al. (2015) obtained 40% COD solubilization by alkaline pre-treatment and ozonation. In the study of Subha and Muthukumar (2012) this release was as high

as 72%. Alkaline pre-treatment also gave the highest COD release efficiency on our ozonation experiment sets as 24.4% at 400 mg O_3/gMLSS. As shown in Table 3.8 and Figure 3.10, COD release almost stopped after 225 mg O3/g MLSS. Inefficiency of excessive sludge ozonation also reported by Subha and Muthukumar (2012) and Semblante et al. (2017). The reason for this can be forming intermediates and by-products that serve as radical scavengers.

Table 3. 8. COD release during ozonation with pH:10 pre-treatment.

COD Release (%)			
	100 mgO_3/gMLSS	**225 mgO_3/gMLSS**	**400 mgO_3/gMLSS**
Mean	17.2	23.9	24.4
Median	16.4	23.9	24.4
Maximum	22.4	27.7	28.7
Minimum	13.3	20.0	20.0

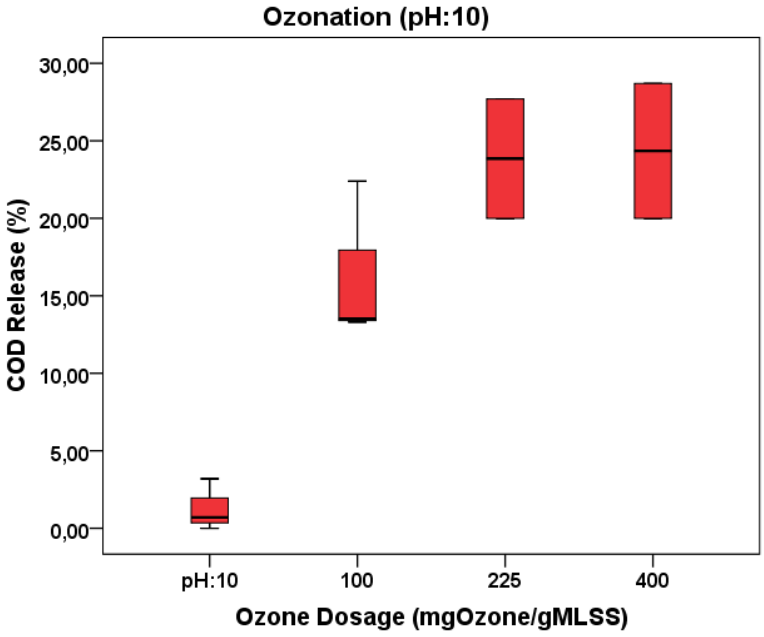


Figure 3. 10. COD release during ozonation with pH:10 pre-treatment.

Inconsistent with Subha and Muthukumar (2012), highest MLSS and MLVSS solubilizations as 48.3% and 59.8% also obtained by alkali pre-treatment and ozonation (Figure 3.11.1 & Table 3.9). Kumar et al (2015) reported 30% MLSS solubilization by

the same combination. The extremely high MLVSS solubilization might be the result of foaming problem during sludge ozonation which shown in Figure 3.11.2, organic loss due to foaming might be mistaken for MLSS and MLVSS solubilization.

Table 3. 9. MLSS and MLVSS solubilizations during ozonation with pH:10 pre-treatment.

MLSS Solubilization (%)	**Ozone Dosage ($mgO_3/gMLSS$)**	**100**	**225**	**400**
	Mean	30.1	35.8	48.3
	Median	21.2	35.8	48.3
	Maximum	59.9	42.0	63.8
	Minimum	9.1	29.7	32.7
MLVSS Solubilization (%)	**Ozone Dosage ($mgO_3/gMLSS$)**	**100**	**225**	**400**
	Mean	45.2	49.0	59.8
	Median	40.9	49.0	59.8
	Maximum	64.2	50.4	68.3
	Minimum	18.5	47.5	51.3

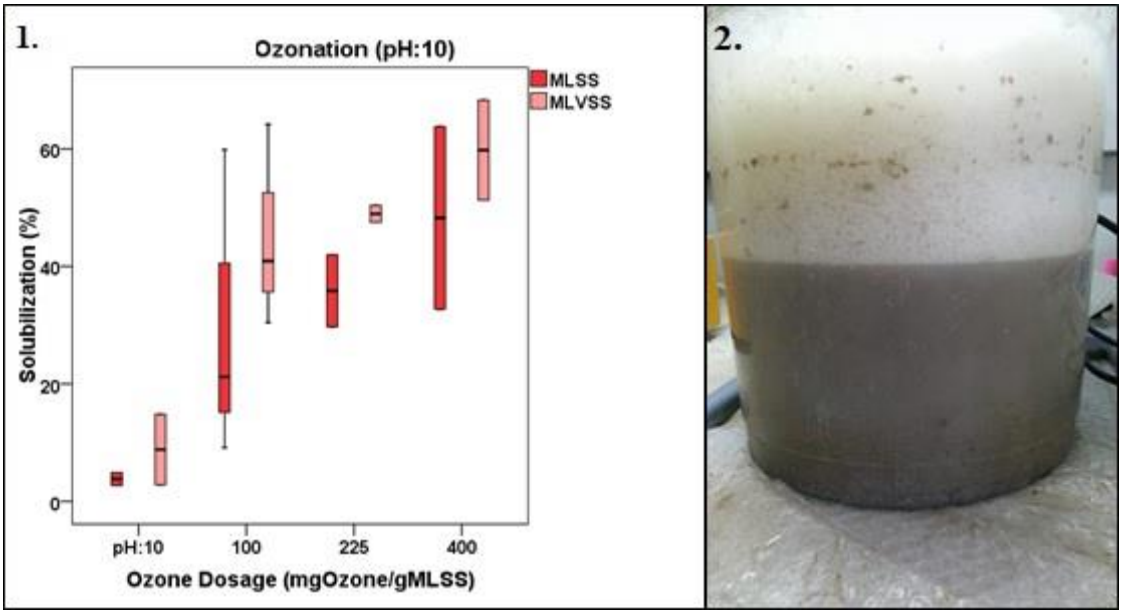


Figure 3. 11. 1. MLSS and MLVSS solubilizations during ozonation with pH:10 pre-treatment**, 2.**Sludge foaming during ozonation with pH:10 pre-treatment.

3.2.4. Sludge solubilization with microwave treatment

Microwave treatment is another effective method for sludge disintegration. Kavitha et al. (2016) have shown that 22% COD release can be obtained by microwave without pre-treatment. Another study that shows the effectiveness of microwave on sludge solubilization was published by Serrano et al., (2016). A 25.9% of COD release was obtained in this study. This release has slightly increased by pH:2 and pH:10 pre-treatments as 27.5% and 26.5% as shown in Figure 3.12 and Table 3.10. In the literature, higher COD releases up to 52.5% have been obtained by alkaline pre-treatment with microwave (Tyagi et al., 2013). These findings have shown the synergistic effect of alkaline and microwave treatments on sludge solubilization. Beszédes et al. (2009) showed that microwave at pH:2 for 5 minutes has nearly same effect on COD release with 60 minutes ozonation. Enhancing effects of acidic agents on microwave treatment of sludge have also been reported by Lee et al. (2015). Although this combination is not recommended at all due to increased operating costs

Table 3. 10. COD releases with microwave after pH pre-treatment.

COD Release (%)				
	neutral	**pH:2**	**pH:4**	**pH:10**
Mean	25.9	27.4	27.5	26.5
Median	28.5	27.4	27.5	24.1
Maximum	32.0	28.3	27.5	36.2
Minimum	17.2	26.6	27.5	19.3

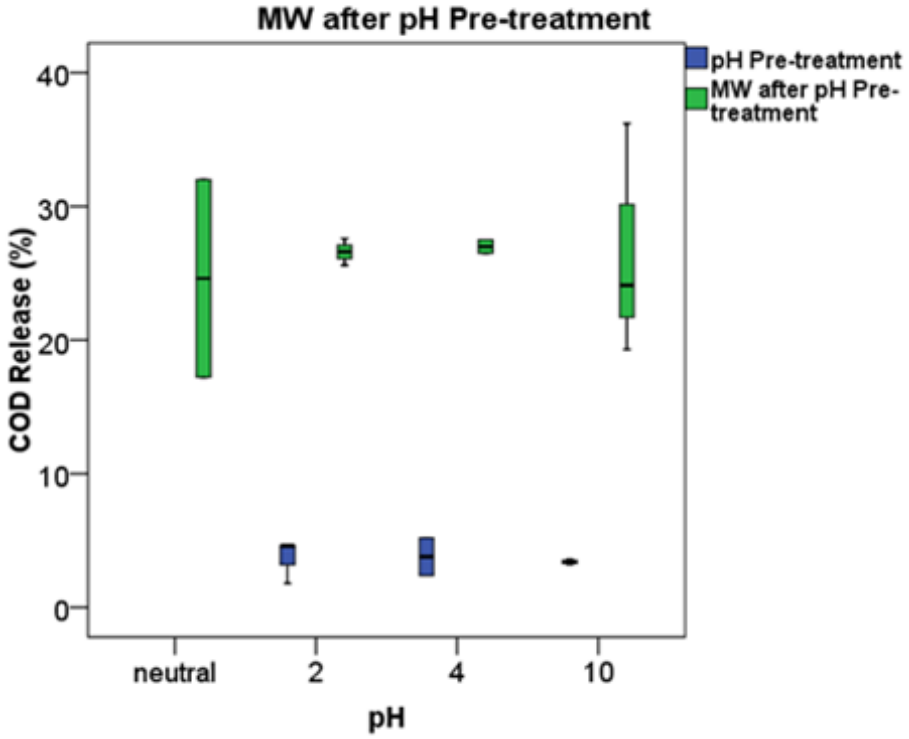


Figure 3. 12. COD releases with microwave after pH pre-treatments.

Higher MLSS and MLVSS releases were also observed with acidic or alkaline pre-treatments than neutral microwave treatment. While microwave experiments without any pre-treatment resulted with 24.5 and 29.5% MLSS and MLVSS solubilizations, this release was increased to 40.9% MLVSS by the help of alkaline pre-treatment (Table 3.11, Figure 3.13). Highest sludge disintegration was obtained as 31.6% MLSS and 46.3 % MLVSS solubilizations in microwave after acid pre-treatment experiments. Varied sludge disintegrations by microwave treatment have been reported in the literature. Kavitha et al. (2016) achieved to 17.33% SS reduction by microwave. 28.5% VSS solubilization has been obtained by Lee at al.(2015) with microwave treatment with acidic pre-treatment. Qiao et al. (2008) reported 40% and 50% VSS dissolution by microwave treatment of neutral and alkaline pre-treated sludges in accordance with our results.

Table 3. 11. MLSS and MLVSS solubilizations with MW after pH pre-treatment.

		neutral	pH:2	pH:10
MLSS Solubilization (%)	**Mean**	24.5	31.6	24.5
	Median	17.7	31.6	24.5
	Maximum	44.6	33.9	48.0
	Minimum	11.1	20.0	20.5
		neutral	**pH:2**	**pH:10**
MLVSS Solubilization (%)	**Mean**	29.5	46.3	40.9
	Median	18.8	44.3	34.5
	Maximum	51.2	75.9	55.0
	Minimum	18.6	18.8	33.3

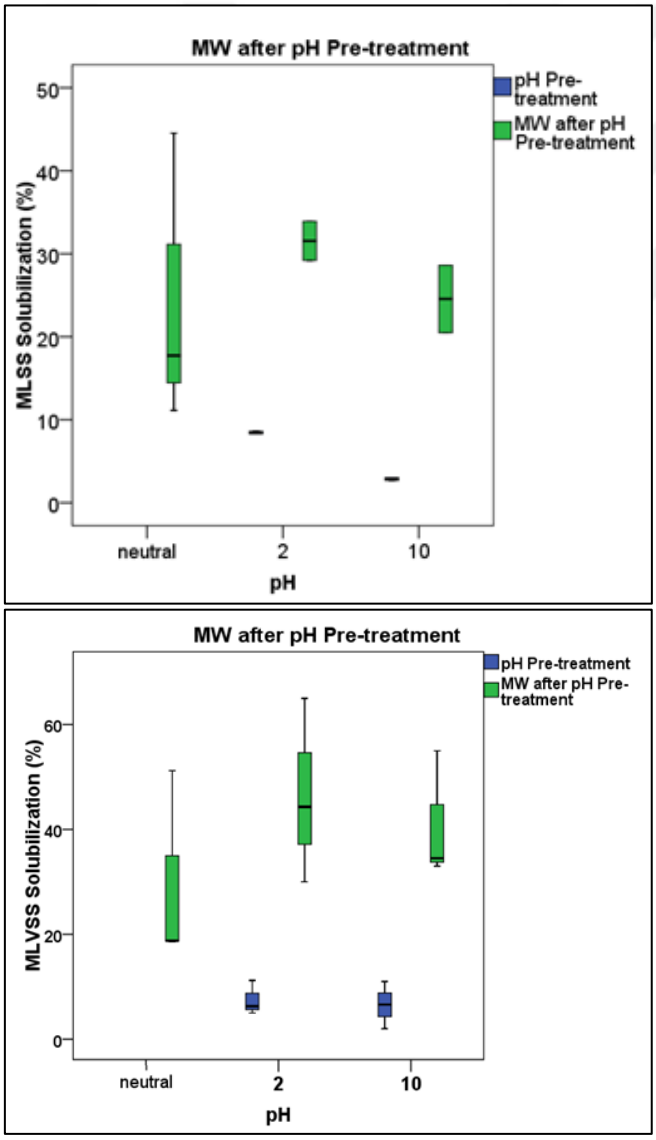


Figure 3. 13. MLSS and MLVSS solubilizations with microwave after pH pre-treatment.

3.2.5. Sludge solubilization in microwave treatment of ozonated sludge samples

Despite lots of studies in the literature about ozonation and microwave, only a few of them have focused on combination of these two tecnologies. One of them has been reported by Beszédes et al. (2009) and shown the combined ozone/microwave treatment enhanced the biodegradability of sludge. Yin et al. (2007) obtained higher (around 24%) COD release by the combination of ozone/microwave than both of these two technologies seperately and this release was enhanced to 37% by additional H_2O_2 pre-treatment. In this study, different pH pre-treatments have been applied with ozonation and microwave to the sludge for enhanced sludge solubilization and nutrient release.

As shown in Figure 3.14 and Table 3.12, microwave treatment of ozonated sludge samples resulted with by far better releases than both ozonation and microwave treatments after pH:2 and pH:10 pre-treatments. While 24.4% COD release was observed at 400 mg O_3/gMLSS ozone dosage and alkaline pre-treatment, by treating this samples by microwave, the COD release have been increased to 43%. This enhancement was much more noticeable on acidic pre-treated sludge. Ozonation of pre-treated sludge samples at pH:2 provided 11.4% COD release while microwave treatment of this samples provides 50.5%. These results show again the effectiveness of acidic pre-treatment on microwave treatment of sludge.

Table 3. 12. COD releases with microwave treatment of ozonated sludge samples.

COD Release (%)						
	pH:2			**pH:10**		
Ozone Dosage (mgO_3/gMLSS)	**100**	**225**	**325**	**100**	**225**	**400**
Mean	34.2	48.0	50.5	30.4	40.7	43.0
Median	34.2	48.0	50.5	29.5	40.7	43.0
Maximum	38.4	50.2	54.2	37.6	41.8	43.0
Minimum	30.0	45.8	46.7	24.1	39.7	43.0

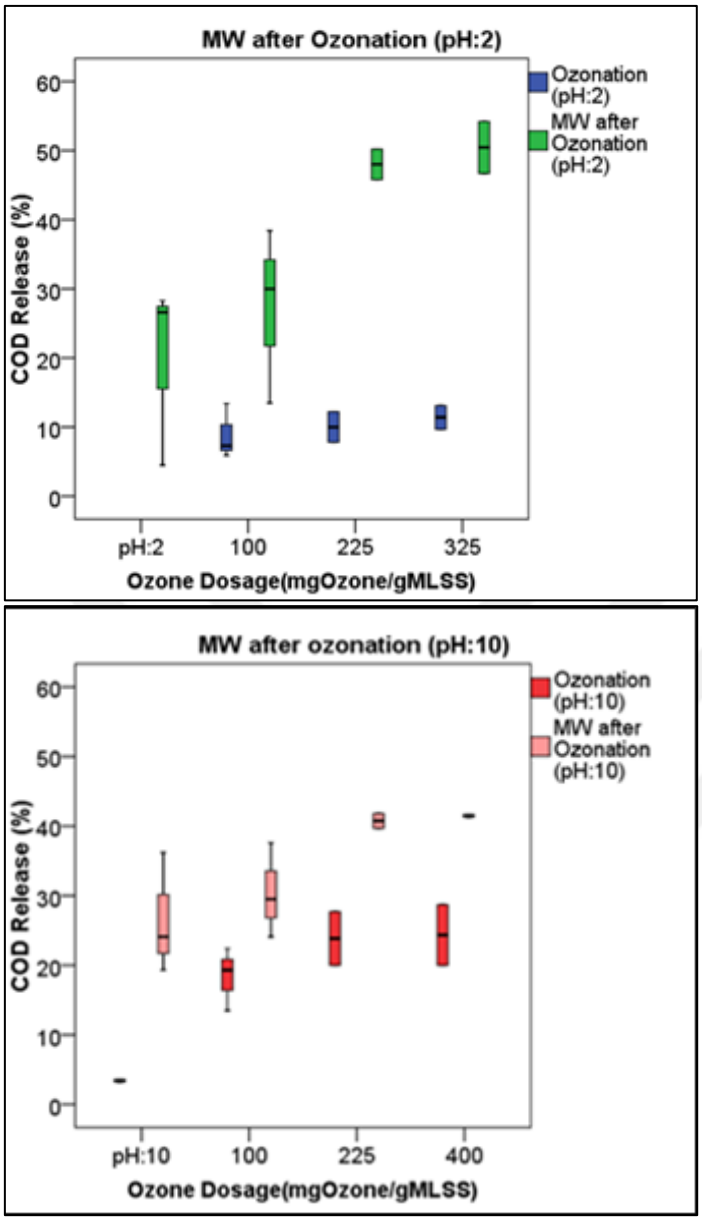


Figure 3. 14. COD releases with microwave treatment of ozonated sludge samples with different pH pre-treatments.

MLSS and MLVSS solubilizations were also varied by microwave treatment. 33.3% and 38.3% MLSS solubilizations were obtained by pH:2 and pH:10 pre-treatments with 100 mg O_3/gMLSS ozonation and microwave (Table 3.13). MLSS and MLVSS solubilizations at 325 mg O_3/gMLSS (pH:2) were increased from 20.7 and 20.3 to 49.5 and 58.7% by microwave treatment. MLSS and MLVSS solubilizations in microwave treatment of alkaline pre-treatment combining ozonation sludge samples increased from 48.3 and 59.8 to 64.5 and 73.5% at 400 mg O_3/gSS. However, as shown in Figure 3.15, higher solubilizations in pH:10/ozontion/microwave experiments were actually results

of higher ozonation efficiencies, the difference between ozonation and microwave experiments were much more noticeable in acidic pre-treated samples.

Table 3. 13. MLSS and MLVSS solubilizations with microwave treatment of ozonated sludge samples.

MLSS Solubilization (%)		**pH:2**			**pH:10**		
	Ozone Dosage ($mgO_3/gMLSS$)	**100**	**225**	**325**	**100**	**225**	**400**
	Mean	33.3	39.3	49.5	38.3	45.8	64.5
	Median	33.3	39.3	49.5	38.3	45.8	64.5
	Maximum	37.3	39.7	58.2	39.1	51.5	72.9
	Minimum	29.2	38.9	40.8	37.6	40.2	56.1
MLVSS Solubilization (%)		**pH:2**			**pH:10**		
	Ozone Dosage ($mgO_3/gMLSS$)	**100**	**225**	**325**	**100**	**225**	**400**
	Mean	47.6	51.5	58.7	46.5	56.3	73.5
	Median	47.6	51.5	58.7	46.5	56.3	73.5
	Maximum	52.8	57.5	70.6	52.8	56.8	77.3
	Minimum	42.4	45.5	46.9	40.3	55.9	69.7

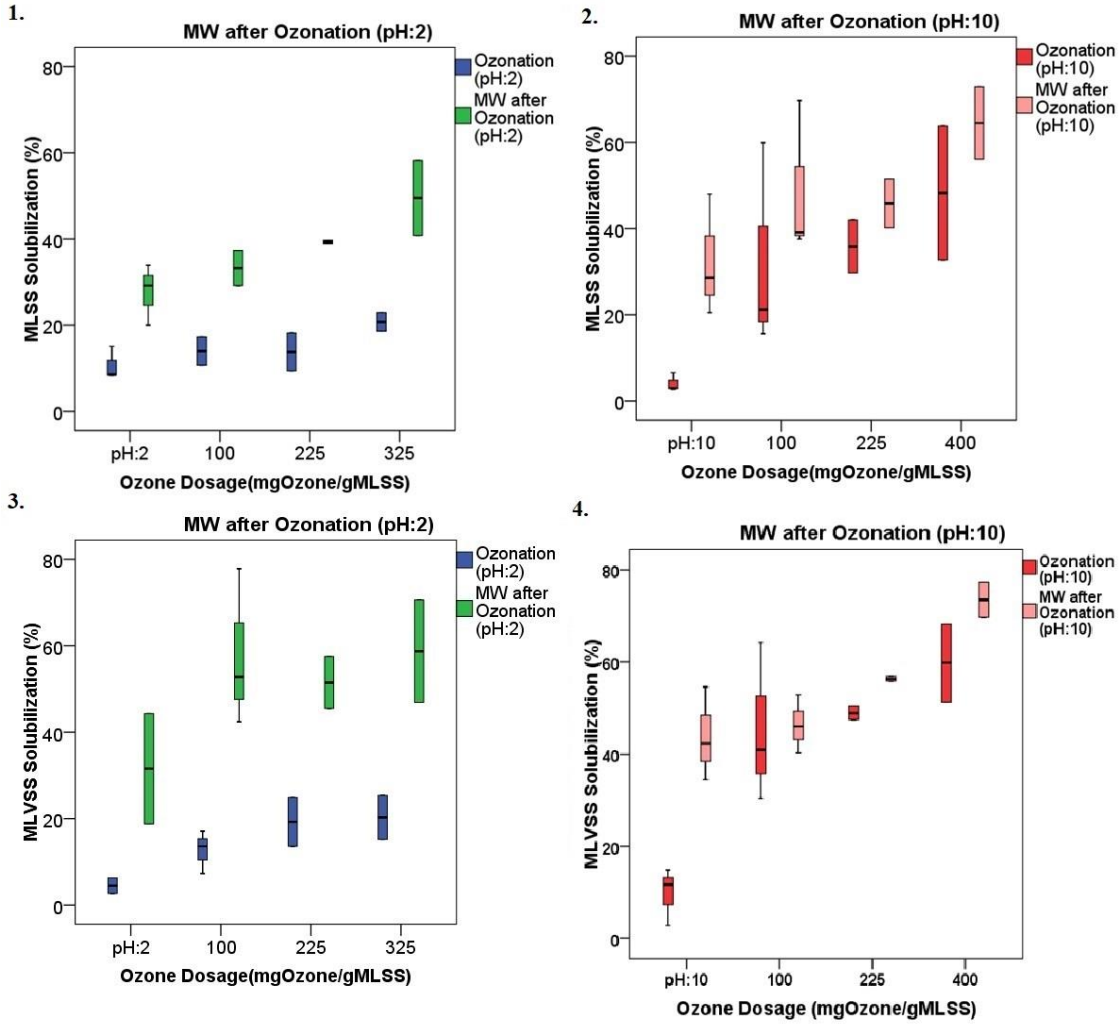


Figure 3. 15. 1. MLSS solubilization with microwave treatment of ozonated sludge samples (pH:2),**2.** MLSS solubilization with microwave treatment of ozonated sludge samples (pH:10), **3.** MLVSS solubilization with microwave treatment of ozonated sludge samples (pH:2), **4.** MLVSS solubilization with microwave treatment of ozonated sludge samples (pH:10).

As an interesting result, an increase in total COD has been observed after microwave treatment of ozonated sludge samples in tune with results of Wang et al. (2016) as shown in Figure 3.16. This might be the result of release of materials such as proteins polysaccharides which could not detected by COD test at first.

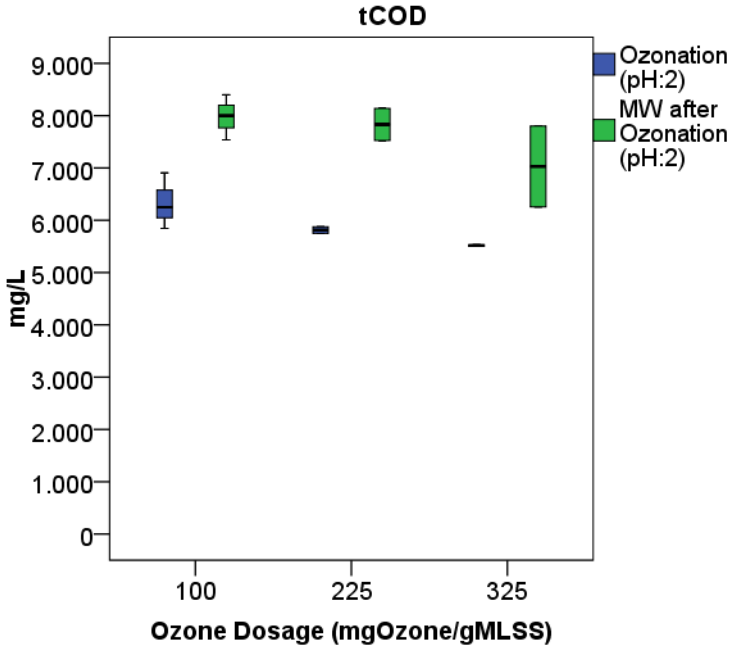


Figure 3. 16. Increase in total COD concentration after microwave treatment.

3.3. Phosphorus Release

Another result of sludge solubilization is phosphorus release, which is the main objective of this study. As mentioned by Saktaywin et al. (2005), the results showed the phosphorus release by solubilization of PAO containing activated sludge. This might be the result of the solubilization of phospholipids of cell membrane and transmission of internal polyphosphates into the solution (Saktaywin et al. 2005 & Cloete and Oosthuizen, 2001).

3.3.1. Phosphorus release with different pH pre-treatments

As shown in Figure 3.17, pH-pretreatments increased the reactive phosphorus concentration in the sludge. Initial PO_4-P concentration was around 1.9 mgPO_4-P/g$MLSS_{initial}$ for both recycle line and aeration tank without any significant difference (p: 0.246), this concentration has been increased to 3.6 & 3.9 and 2.4 by pH:2, pH:4 and pH:10 pre-treatments. These results, in line with Xu et al. (2015), showed that acidic pre-treatment is more effective than alkaline for phosphorus release possibly due to dissolution of inorganics like dicalcium phosphate, octocalcium phosphate, newberyite, and struvite (Zhang et al., 2014) in addition to cell lysis. Also, bigger portion of the organic and acid hydrolyzable phosphorus was released by acidic pre-treatment than alkaline.

Table 3. 14. Phosphorus release with different pH pre-treatments.

Phosphorus ($mgPO_4$-P/$gMLSS_{initial}$)			
	pH:2	**pH:4**	**pH:10**
Mean	3.6	3.9	2.4
Median	3.6	3.9	2.0
Maximum	6.9	4.3	3.6
Minimum	1.5	3.4	1.0

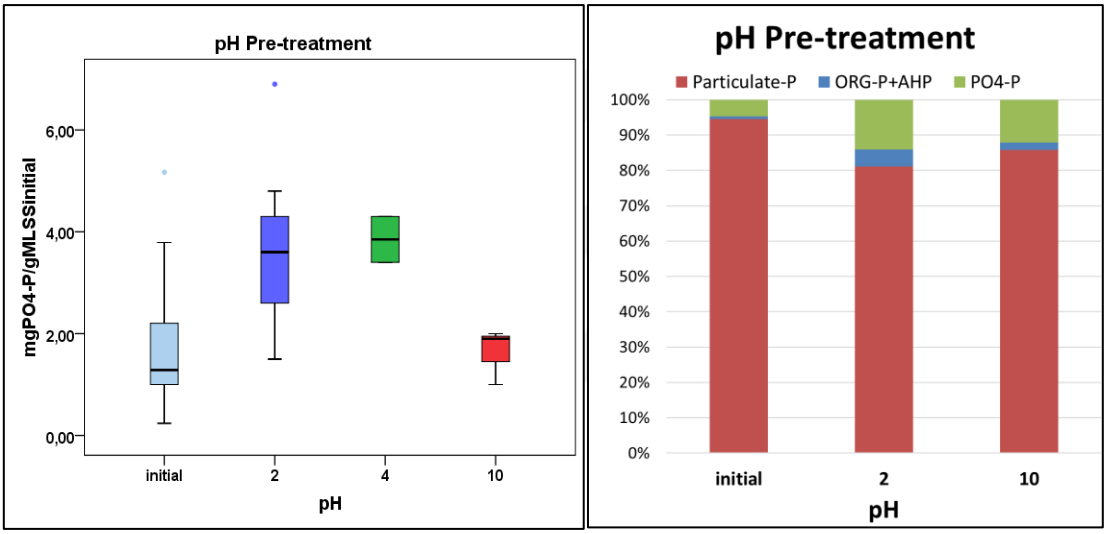


Figure 3. 17. Phosphorus release with different pH pre-treatments.

3.3.2. Phosphorus release with ozonation

As shown in Table 3.15 and Figure 3.18, ozonation without any pre-treatment was almost ineffective for phosphorus recovery when comparing with other methods. While initial phosphorus content was 1.9 $mgPO_4$-P/$gMLSS_{initial}$, ozonation increased this number to 3.6 $mgPO_4$-P/$gMLSS_{initial}$. Phosphorus behavior during ozonation was quite similar to results of Saktaywin et al. (2005), reactive phosphorus concentration quickly increased at the beginning of the ozonation and then nearly stabilized. On the other hand, in the study of Quiang et al. (2015) ozonation increased the phosphorus concentration from 0.38 to 5.63 $mgPO_4$-P/$gMLSS_{initial}$ with higher efficiency.

Table 3. 15. Phosphorus release during ozonation.

Phosphorus ($mgPO_4$-P/$gMLSS_{initial}$)				
	50 mgO_3/gMLSS	**100 mgO_3/gMLSS**	**150 mgO_3/gMLSS**	**250 mgO_3/gMLSS**
Mean	3.0	3.1	3.0	3.6
Median	3.4	3.2	2.8	3.5
Maximum	3.8	4.4	4.5	3.9
Minimum	1.0	1.9	1.7	3.4

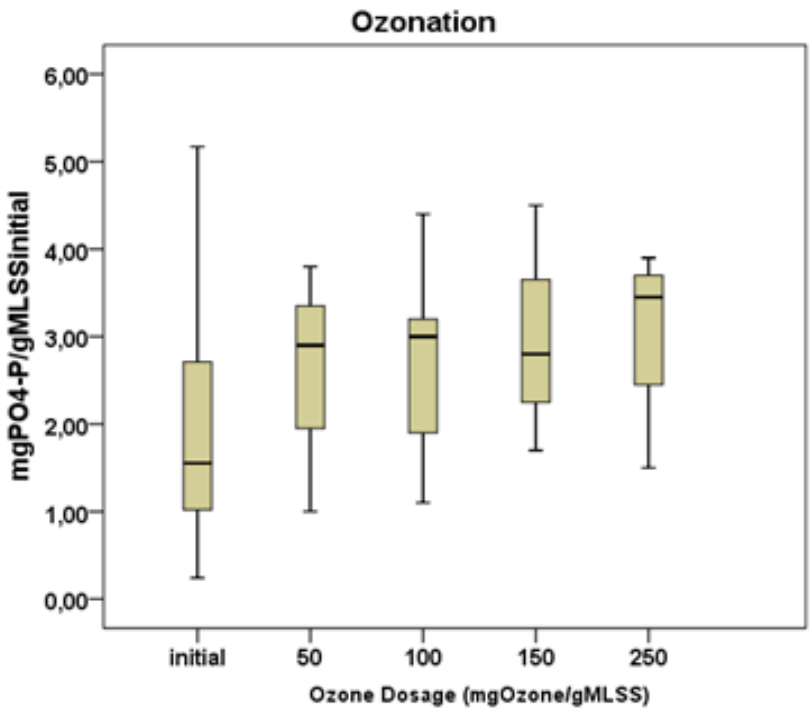


Figure 3. 18. Phosphorus release during ozonation.

As shown in Figure 3.19 and Table 3.16, acidic pre-treatment slightly enhanced the reactive phosphorus release (p: 0.037). While 3.6 $mgPO_4$-P/$gMLSS_{initial}$ was obtained by ozonation at 250 mg O_3/gMLSS, higher amount of reactive phosphorus was observed at lower dosages by additional acid pre-treatments. It shows a possible decrease for operational cost of phosphorus recovery by ozonation.

Table 3. 16. Phosphorus release with ozonation & acid pre-treatments.

Phosphorus ($mgPO_4$-P/$gMLSS_{initial}$)							
	pH:2				pH:4		
Ozone Dosage (mgO_3/gMLSS)	**50**	**100**	**225**	**325**	**100**	**175**	**300**
Mean	3.2	3.5	3.7	4.7	4.3	4.5	4.7
Median	3.1	3.2	3.7	4.7	4.3	4.5	4.7
Maximum	5.5	5.7	4.4	6.2	4.3	4.8	5.1
Minimum	1.8	1.9	2.9	3.2	4.3	4.2	4.3

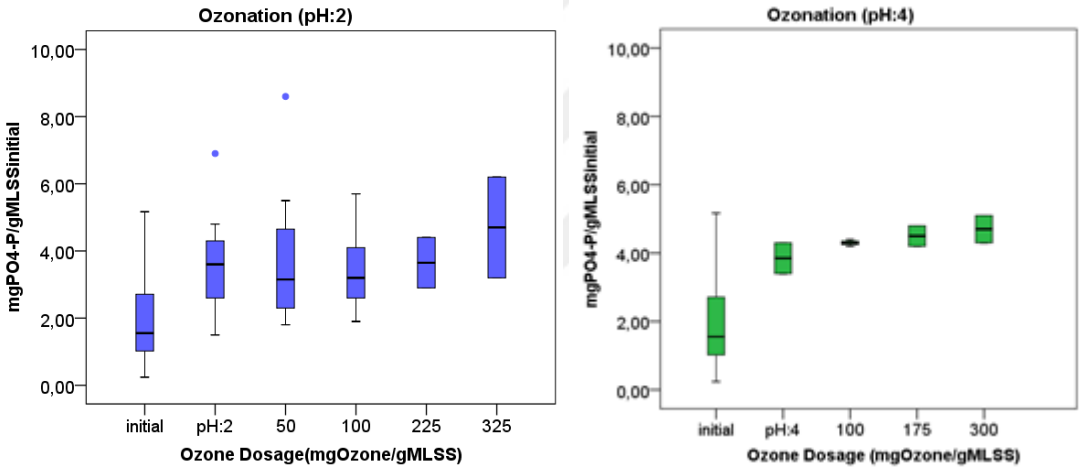


Figure 3. 19. Phosphorus release with ozonation & acidic pre-treatments.

Examination of phosphorus fractions during ozonation gave more detailed information about behavior of phosphorus. As shown in Figure 3.20, 21% of particulate phosphorus released into the solution at 325 mg O_3/gMLSS ozone dosage, and some part of organic and acid hydrolyzable phosphorus released together with PO_4-P as mentioned in Saktaywin et al. (2005).

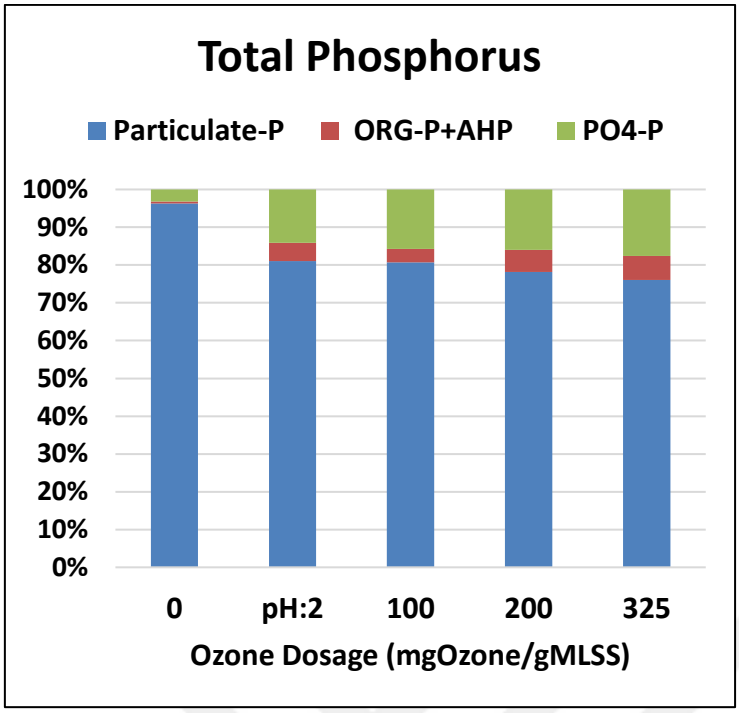


Figure 3. 20. Phosphorus fraction after ozonation & pH:2 pre-treatment.

As shown in Figure 3.21 and Table 3.17 alkaline pre-treatment showed the highest release of phosphorus with ozonation (p:0.043), but there were no significant differences statistically between pH:2 and pH:10 pre-treatments on phosphorus release by ozonation (p: 0.816). At 225 mg O_3/gMLSS phosphorus content of sludge increased to 4.8 mgPO_4-P/gMLSS$_{initial}$, and then decreased to 3.7 at 400 mg O_3/gMLSS. The reason for this may be phosphorus precipitation due to high pH. Alkaline pre-treatment was also more effective on releasing organic and acid hydrolyzable phosphorus besides reactive phosphorus. As shown in Figure 3.21 30% of particulate phosphorus solubilized after 200 mg O_3/gMLSS ozone dosage.

Table 3. 17. Phosphorus release during ozonation &pH:10 pre-treatment.

Phosphorus (mgPO_4-P/gMLSS$_{initial}$)			
	100 mgO_3/gMLSS	**225 mgO_3/gMLSS**	**400 mgO_3/gMLSS**
Mean	3.3	4.8	3.7
Median	3.7	4.8	3.7
Maximum	4.0	5.4	5.6
Minimum	1.6	4.2	1.8

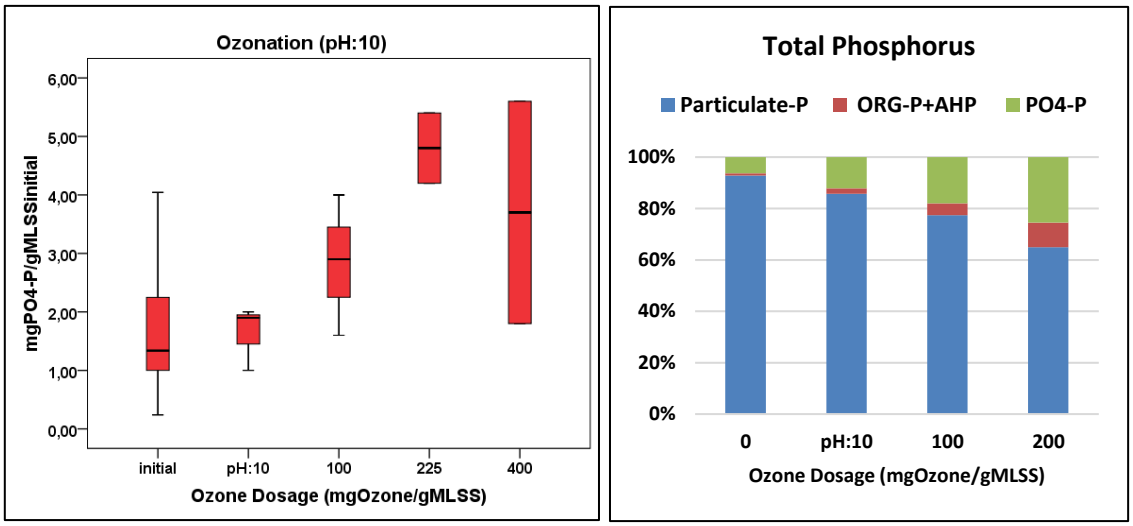


Figure 3. 21. Phosphorus release during ozonation &pH:10 pre-treatment**.**

3.3.3. Phosphorus release with microwave treatment

As a result of cell lysis, microwave treatment increases the reactive phosphorus in sludge, destroys cells and hardly degradable organics. Microwave irradiation causes the release of internal polyphosphates and phosphorus in extracellular polymeric substanceses, and up to 96% of total phosphorus might be solubilized into the solution (Tyagi et al., 2013). Short term treatments even as 5 minutes might provide 76% release of total phosphorus as reported by Liao et al. (2005). Wang et al. (2015) applied microwave and obtained a high increase in PO_4-P concentration around 275%. In this study, the increase in reactive phosphorus by microwave was around 174% without any pre-treatment. Reactive phosphorus content of sludge, which was initially 1.9 mgPO_4-P/gMLSS$_{initial}$, was increased to 5.2 mgPO_4-P/gMLSS$_{initial}$ by microwave treatment. However, in contrast to the study of Wang et al. (2015) -which propose the acidic and alkaline pre-treatments are almost ineffective- this release was enhanced by pH pre-treatments. Effects of pH pre-treatments on phosphorus release by microwave was also reported by the following study of the same author (Wang et al., 2016). In this study, by acid and alkaline pre-treatments, reactive phosphorus concentrations were increased to 10.9, 9 and 6.3 mgPO_4-P/gMLSS$_{initial}$ at pH:2, pH:4 and pH:10 (Table 3.18& Figure 3.22). Similar to Wang et al. (2016), acidic pre-treatments were more effective than alkaline on both reactive and nonreactive soluble phosphorus release.

Table 3. 18. Phosphorus release with microwave & different pH pre-treatments.

Phosphorus ($mgPO_4$-P/$gMLSS_{initial}$)				
	neutral	**pH:2**	**pH:4**	**pH:10**
Mean	5.2	10.9	9.0	6.3
Median	6.2	10.9	9.0	6.4
Maximum	6.4	11.3	11.2	7.5
Minimum	3.1	10.5	6.8	5.0

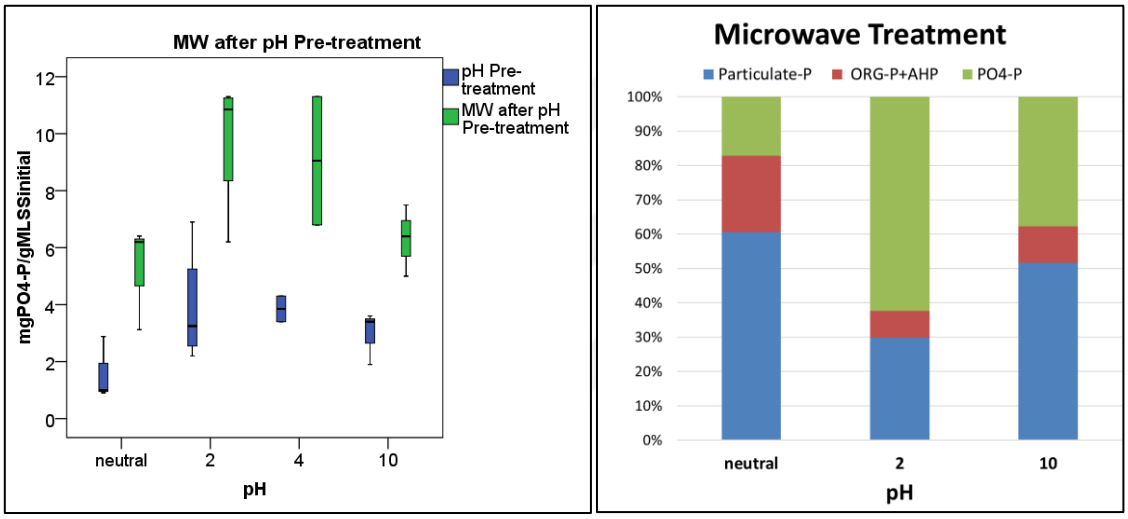


Figure 3. 22. Phosphorus release with microwave treatment.

3.3.4. Phosphorus release with microwave treatment of ozonated sludge samples

Highest phosphorus releases were obtained by microwave treatment of ozonated sludge samples. As shown in Table 3.19 and Figure 3.23 acid pre-treatment/ozonation/ microwave experiments provided high phosphorus contents as 13.4 and 12.9 $mgPO_4$-P/$gMLSS_{initial}$ at pH:2 and pH:4. These values are nearly equivalent to 1.3% of sludge and 65% of total phosphorus.

Table 3. 19. Phosphorus release with microwave treatment of ozonated sludge samples (pH:2&pH:4)

Phosphorus ($mgPO_4$-P/$gMLSS_{initial}$)						
	pH:2			pH:4		
Ozone Dosage (mgO_3/gMLSS)	100	225	325	175	300	450
Mean	11.2	12.9	13.4	10.4	11.4	12.9
Median	11.2	12.9	13.4	10.4	11.4	12.9
Maximum	11.8	13.8	13.9	12.1	12.8	12.9
Minimum	10.5	12.0	12.8	8.6	10.1	12.9

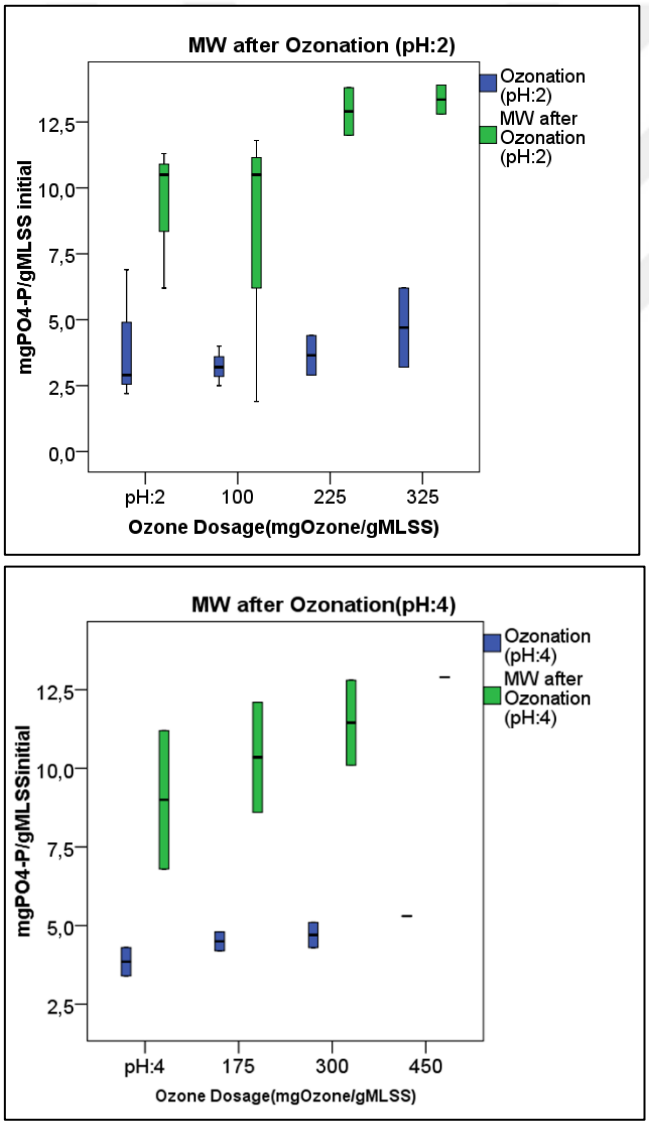


Figure 3. 23. Phosphorus release by microwave treatment of ozonated sludge samples with acidic pre-treatments.

Determining of phosphorus fraction after combining ozonation and microwave with acidic pre-treatment showed that most of particulate phosphorus solubilized as shown in Figure 3.24.

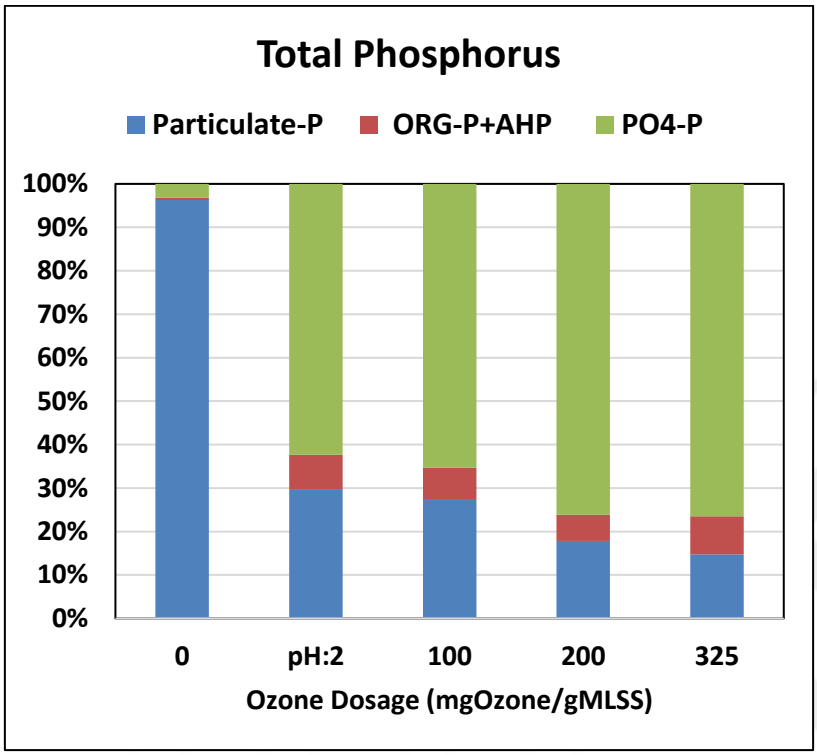


Figure 3. 24. Phosphorus fraction after microwave treatment of ozonated sludge (pH:2).

Highest reactive phosphorus concentration which was obtained by combining alkaline pre-treatment/ozonation/ microwave treatment was also 10,9 $mgPO_4$-$P/gMLSS_{initial}$ at 225 mg O_3/gMLSS. After this dosage, excessive ozonation caused a decrease to 10.4 $mgPO_4$-$P/gMLSS_{initial}$ as shown in Figure 3.25 and Table 3.20. While considering the total phosphorus content of our sludge samples were around 2% and higher phosphorus releases in the literature, these numbers might be the upper limit for these experimental conditions. As mentioned before, 10.9 $mgPO_4$-$P/gMLSS_{initial}$ have been obtained by microwave treatment directly after acidic pre-treatment (Figure 3. 25.3) without any significant differences from pH:2/ozonation/microwave treatments (p:0.083). This could be an alternative for phosphorus recovery from activated sludge.

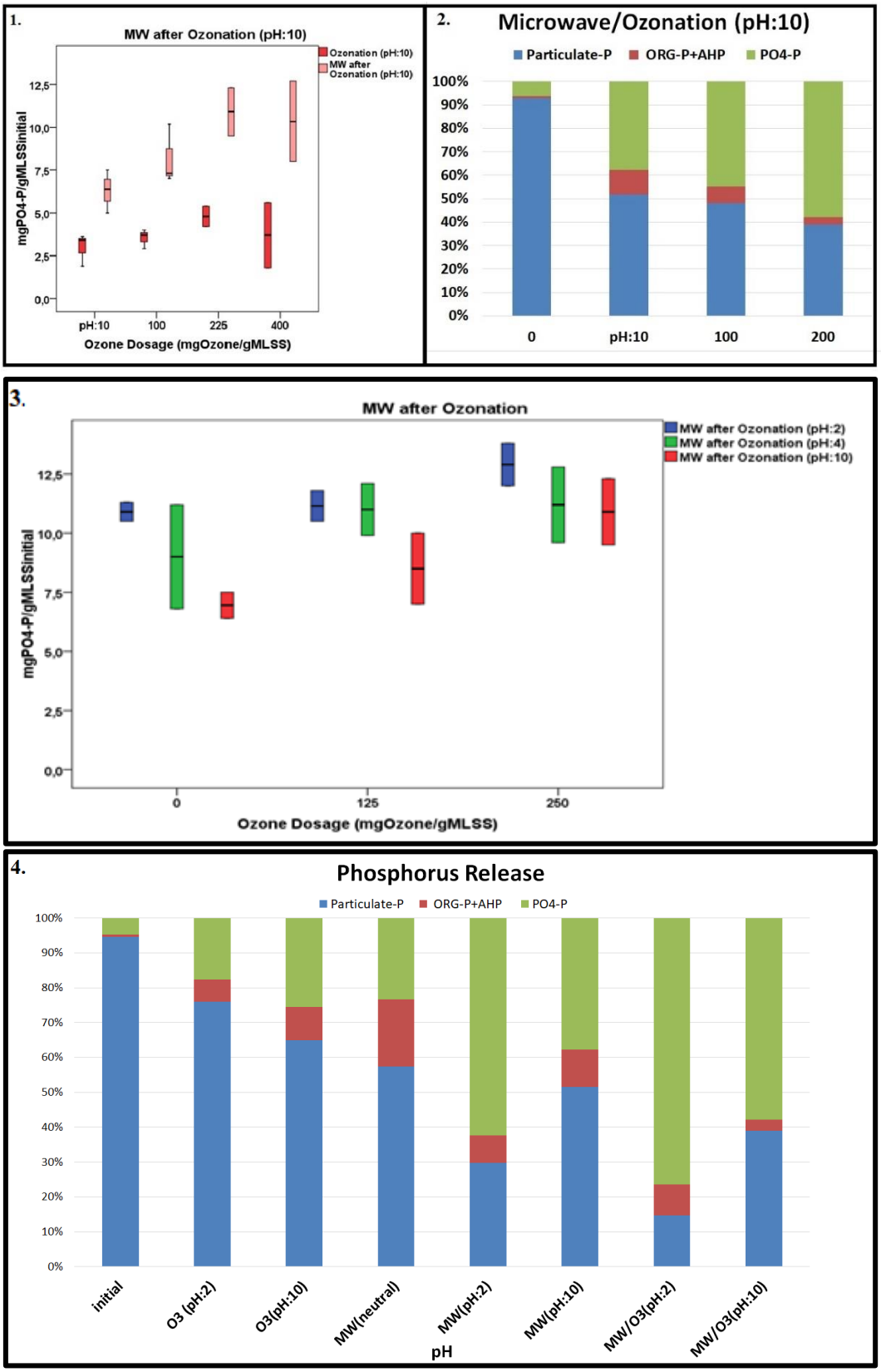


Figure 3. 25. 1. Phosphorus release with microwave treatment of ozonated sludge samples (pH:10), **2.**Phosphorus fraction after microwave treatment of ozonated sludge samples (pH:10**),** .**3.** Overall phosphorus release with microwave treatment of ozonated sludge samples.**4.** Overall phosphorus fractions.

Table 3. 20. Phosphorus release with microwave treatment of ozonated sludge samples (pH:10).

Phosphorus ($mgPO_4$-P/$gMLSS_{initial}$)			
	100 mgO_3/gMLSS	**225 mgO_3/gMLSS**	**400 mgO_3/gMLSS**
Mean	8.2	10.9	10.4
Median	7.3	10.9	10.4
Maximum	10.2	12.3	12.7
Minimum	6.9	9.5	8.0

3.4. Ammonia Release

3.4.1. Ammonia release in different pH pre-treatments

The NH_4-N concentration increased rapidly at low and high pH levels. Acidic and alkaline pre-treatments caused to up to 87% release in ammonia concentration of sludge. While initial ammonia content of sludge was 0.27 $mgNH_4$-$N/gMLSS_{initial,}$ after acidic and alkaline pre-treatments this number increased to 0.45 and 0.51 $mgNH_4$-$N/gMLSS_{initial}$ (Table 3.21 and Figure 3.26). Chen et al. (2007) proposed that acidic pH levels are quite efficient for ammonia release while alkaline is ineffective in their long-term fermentation experiments, possibly due to inhibitory effect of strong alkaline conditions to sludge hydrolytic enzymes like peptidase, protease, etc. On the other hand, Kim et al. (2015) reported higher ammonia release under alkaline conditions. Bi et al. (2014) also showed the ammonia release under alkaline conditions at short term reactions, mainly due to the hydrolysis of nitrogenous organics, such as protein.

Table 3. 21. Ammonia releases with diffferent pH pre-treatments.

Ammonia ($mgNH_4$-$N/gMLSS_{initial}$)			
	pH:2	**pH:4**	**pH:10**
Mean	0.45	0.45	0.51
Median	0.39	0.45	0.44
Maximum	1.22	0.69	0.86
Minimum	0.19	0.22	0.28

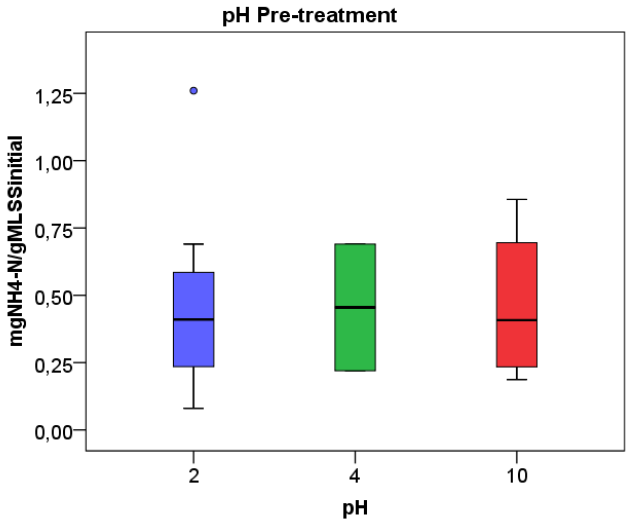


Figure 3. 26. Ammonia releases with different pH pre-treatments.

3.4.2. Ammonia release with ozonation

Another nutrient which releases by ozone oxidation is nitrogen. During ozonation experiments, NH_4-N release observed while NO_3-N concentrations were too low to detect. The increase in ammonia concentration was possibly due to the oxidizing organic nitrogen during ozonation as mentioned by Semblante et al. (2017). Sui et al. (2014) proposed that, at 80-100 mg O_3/gSS ozone dosages 35% of particulate nitrogen released into the solution while only 2% of soluble nitrogen was in the NO_x form at most. In our study, ammonia concentration of sludge increased from 0.27 mgNH_4-N/gMLSS$_{initial}$ to 0.88 mgNH_4-N/gMLSS$_{initial}$ at 100 mg O_3/gMLSS and increased to 1.77 at 225 mg O_3/gMLSS (Table 3.22 and Figure 3.27).

Table 3. 22. Ammonia release during ozonation.

Ammonia (mgNH_4-N/gMLSS$_{initial}$)				
	50 mgO_3/gMLSS	**100 mgO_3/gMLSS**	**150 mgO_3/gMLSS**	**225 mgO_3/gMLSS**
Mean	0.77	0.88	1.49	1.77
Median	0.57	0.73	1.45	2.23
Maximum	1.49	1.84	2.09	2.45
Minimum	0.43	0.12	0.94	0.60

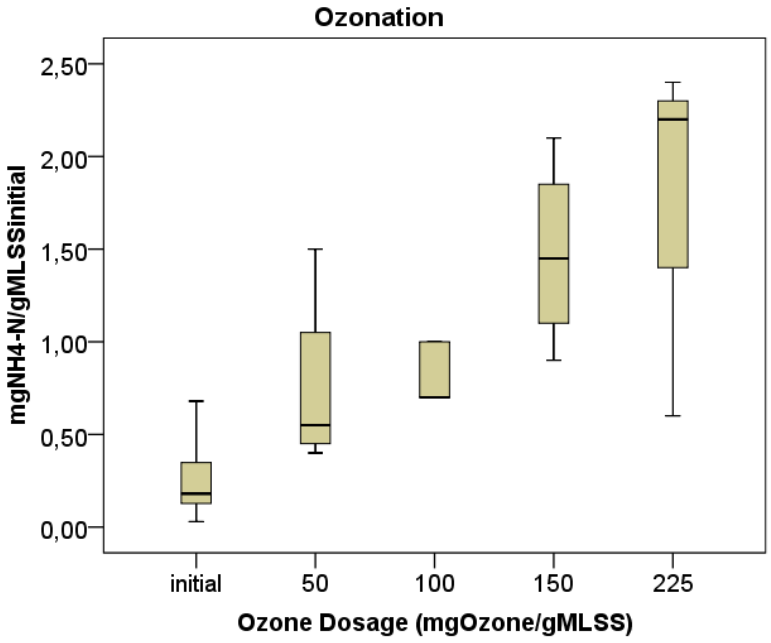


Figure 3. 27. Ammonia release during ozonation.

As shown in Figure 3.28 and Table 3.23, acidic pre-treatment had not a significant effect on ammonia release during ozonation. While ammonia concentration of sludge was 0.88 $mgNH_4\text{-}N/gMLSS_{initial}$ at 100 mg O_3/gMLSS at neutral condition, this value was increased to 1.01 by pH:2 pre-treatment. However, as ozonation continues, the gap between ammonia releases closed. At 225 mg O_3/gMLSS, both neutral and pH:2 ozonation experiments resulted with the same concentration as 1.77 $mgNH_4\text{-}N/gMLSS_{initial.}$

Table 3. 23. Ammonia releases during ozonation with pH:2 pre-treatment.

Ammonia ($mgNH_4\text{-}N/gMLSS_{initial}$)				
	pH:2			
Ozone Dosage (mgO_3/gMLSS)	**50**	**100**	**225**	**325**
Mean	0.79	1.01	1.77	2.52
Median	0.62	0.88	1.77	2.52
Maximum	2.18	1.44	2.00	3.11
Minimum	0.38	0.47	1.53	1.92

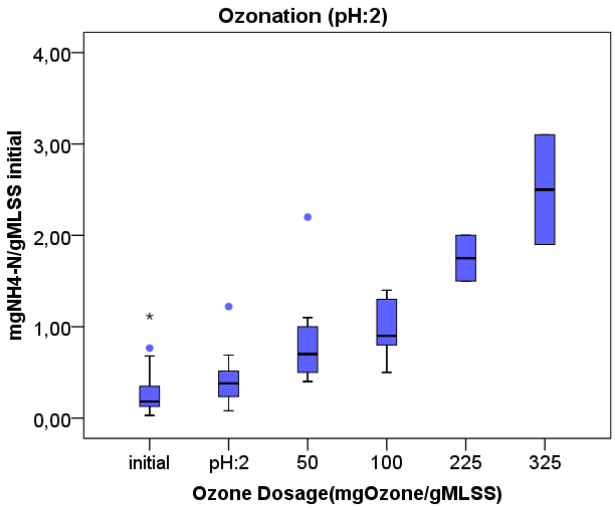


Figure 3. 28. Ammonia release during ozonation with pH:2 pre-treatment**.**

Ammonia releases during ozonation were enhanced by alkaline pre-treatments at lower dosages but fallen behind the both acid pre-treated and neutral experiments as ozonation continues, possibly due to struvite precipitation. Ammonia concentration of sludge increased from 0.27 $mgNH_4\text{-}N/gMLSS_{initial}$ to 1.21 $mgNH_4\text{-}N/gMLSS_{initial}$ at 100 mg O_3/gMLSS (Figure 3.29 and Table 3.24). This numbers refers to 333% increase in ammonia concentration, which almost same percent and same dosage reported by Qiang et al. (2015).

Table 3. 24. Ammonia release during ozonation with pH:10 pre-treatment experimetns.

Ammonia ($mgNH_4\text{-}N/gMLSS_{initial}$)			
	100 mgO_3/gMLSS	**225 mgO_3/gMLSS**	**400 mgO_3/gMLSS**
Mean	1.21	1.32	1.42
Median	1.29	1.32	1.42
Maximum	1.34	1.40	1.42
Minimum	0.92	1.24	1.42

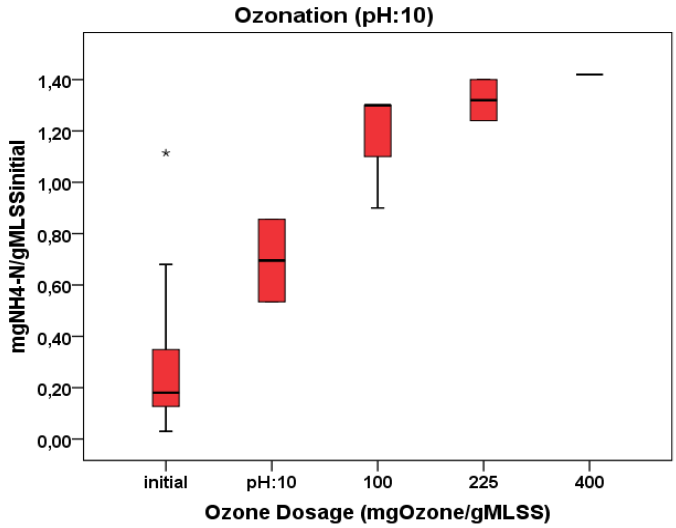


Figure 3. 29. Ammonia release during ozonation with pH:10 pre-treatment**.**

3.4.3. Ammonia release with microwave treatment

Lots of studies in the literature showed that, microwave irradiation is an effective technology for also release of ammonia from excess sludge. Liao et al. (2005) showed the release of ammonia besides the orthophosphate even at relatively low temperatures as 60ºC. Kenge et al. (2009) reported 56% increase in ammonia concentration of sludge by pH:4 pre-treatment and microwave. As shown in Figure 3.30, both acidic and alkaline pre-treatments enhanced the release efficiency of ammonia by microwave. These results conform the findings of Wang et al. (2016) except the higher efficiency of alkaline pre-treatment than acidic. In our study, microwave experiments with alkaline pre-treatment have been resulted with 1.55 $mgNH_4\text{-}N/gMLSS_{initial}$ while acidic pre-treatment with microwave experiments increased the ammonia concentration to 1.46 $mgNH_4\text{-}N/gMLSS_{initial}$.

Table 3. 25. Ammonia release with microwave treatment.

Ammonia ($mgNH_4\text{-}N/gMLSS_{initial}$)			
	neutral	**pH:2**	**pH:10**
Mean	0.74	1.46	1.55
Median	0.74	1.49	1.55
Maximum	0.80	1.59	1.96
Minimum	0.68	1.33	1.14

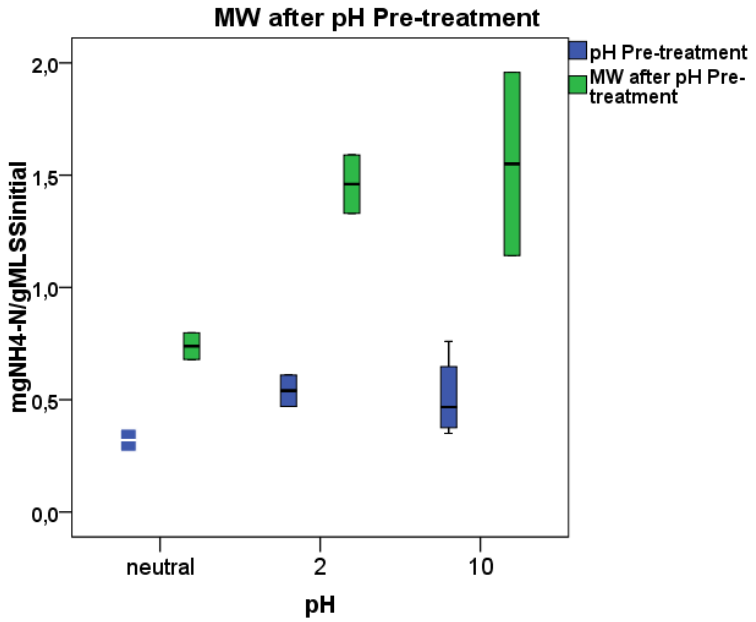


Figure 3. 30. Ammonia release with microwave treatment.

3.4.4. Ammonia release in microwave treatment of ozonated sludge samples

As observed in sludge solubilization and phosphorus release results, highest ammonia concentrations were also obtained by microwave treatment of ozonated sludge samples. While 3.3 $mgNH_4\text{-}N/gMLSS_{initial}$ ammonia concentration was achieved by microwave after 225 $mgO_3/gMLSS$ ozonation with pH:2 pre-treatment, ammonia concentration after microwave with the same ozone dosage and pH:10 pre-treatment was 2.43 $mgNH_4\text{-}N/gMLSS_{initial}$ (Figure 3.31). The effect of high pH levels on ammonia release, was not obtained in microwave treatment of ozonated samples. A possible reason for this result can be struvite precipitation during ozonation as mentioned in Section 3.3.2. Yin et al. (2007) also reported the higher efficiency of combined microwave and ozone treatment than their individual effects for ammonia release from sewage sludge.

Table 3. 26. Ammonia release with microwave treatment of ozonated sludge samples (pH:2 & pH:10).

Ammonia ($mgNH_4$-$N/gMLSS_{initial}$)						
	pH:2			pH:10		
Ozone Dosage ($mgO_3/gMLSS$)	100	225	325	100	225	400
Mean	2.15	3.30	4.13	1.79	2.43	2.49
Median	2.15	3.30	4.13	1.79	2.43	2.49
Maximum	2.22	3.43	4.29	2.12	2.69	2.49
Minimum	2.08	3.17	4.00	1.46	2.16	2.49

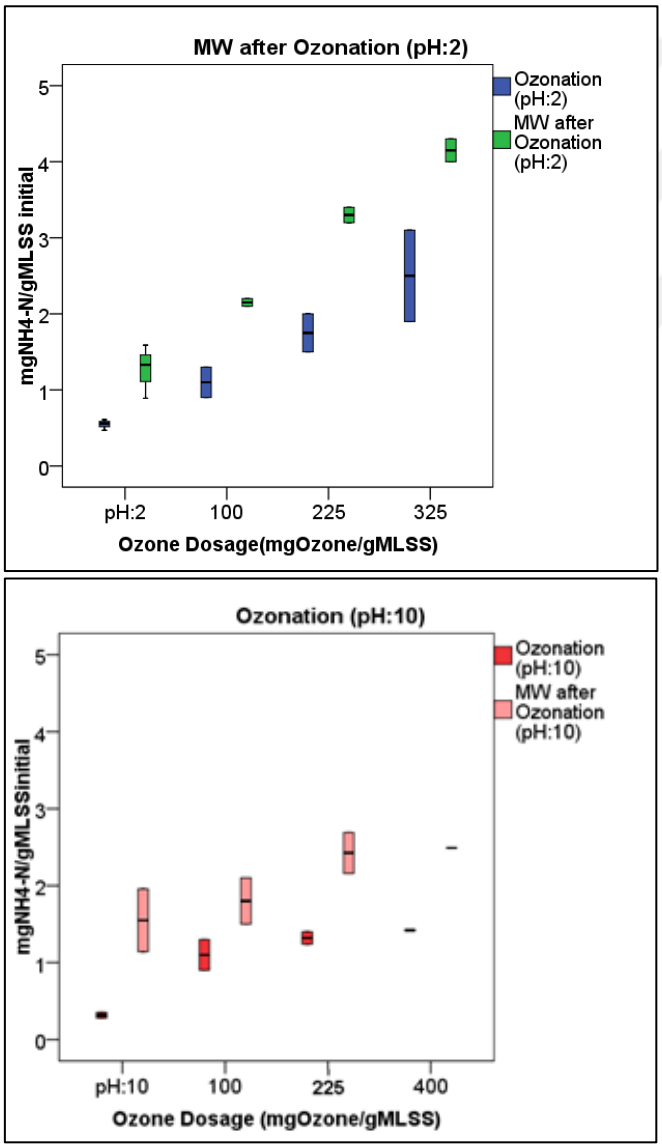


Figure 3. 31. Ammonia releases by microwave treatment of ozonated sludge samples with different pH pre-treatments.

3.5. Behavior of Magnesium and Calcium

As shown in Figure 3.32, magnesium and calcium showed different trends during ozonation. While magnesium concentration was increasing, a decrease in calcium was observed. A possible reason for the decrease of calcium can be precipitation with phosphorus as proposed in the study of Sui et al. (2011), which also mentioned about release of magnesium by ozonation due to cell lysis.

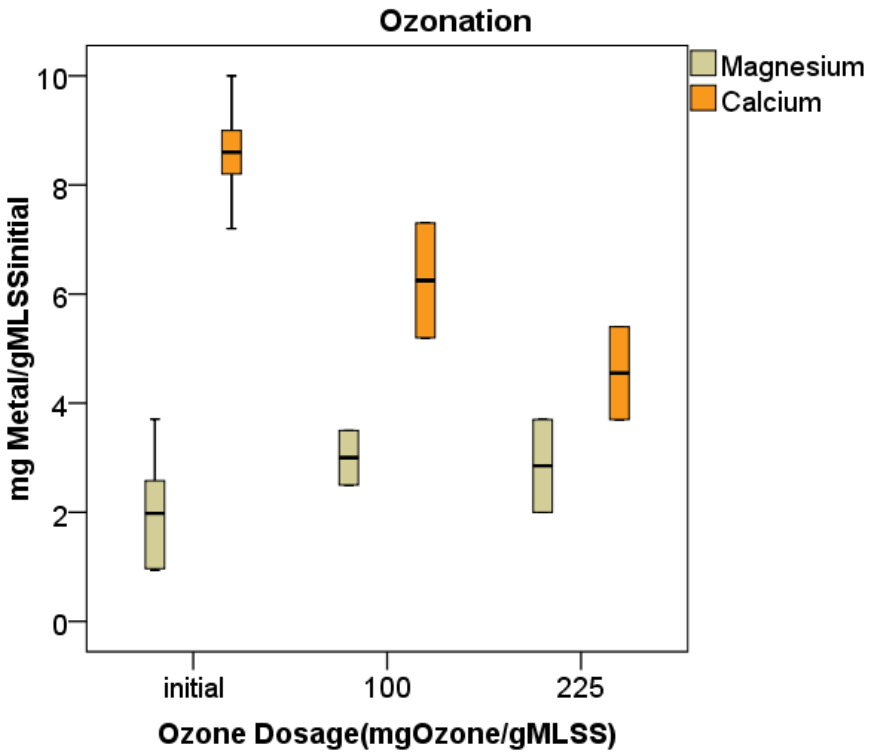


Figure 3. 32. Behaviour of magnesium and calcium during ozonation.

Ozonation with acidic pre-treatment experiments resulted with almost the same consequences. While magnesium was releasing with increasing ozone dosages, calcium concentration decreased again after a short increasing period (Figure 3.33). This short Ca releasing phase possibly was a result of the dissolution of precipitates under acidic condition, not an influence of ozonation.

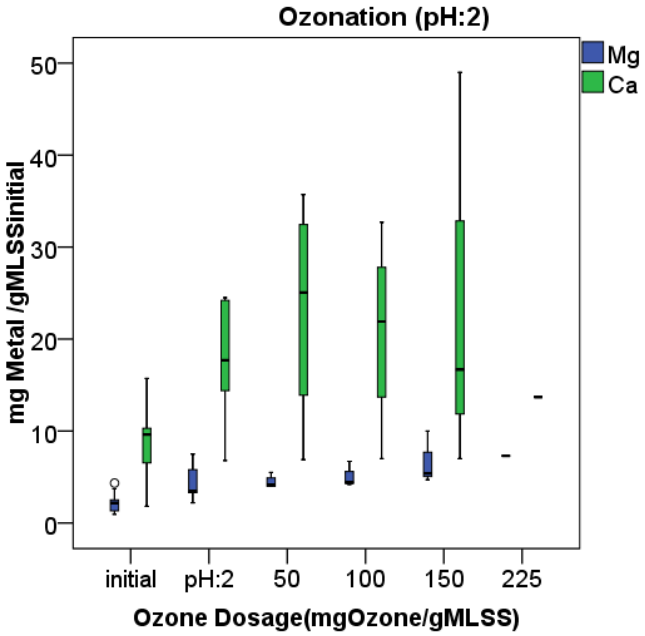


Figure 3. 33. Behaviour of magnesium and calcium during ozonation (pH:2).

Alkaline pre-treatment also effected the behavior of magnesium and calcium during ozonation. An increase in magnesium concentration was observed again, but far less than ozonation of acid pre-treated samples. When considering the ammonia and phosphorus release in ozonation of alkaline pre-treated sludge samples, the results showed again the struvite precipitation during ozonation. On the other hand, calcium concentration decreased at pH:10 pre-treatment and kept continue to decreasing until the high ozone dosages as 400mgO_3/gMLSS (Figure 3.34). This can be the result of precipitation of apatites besides the struvite.

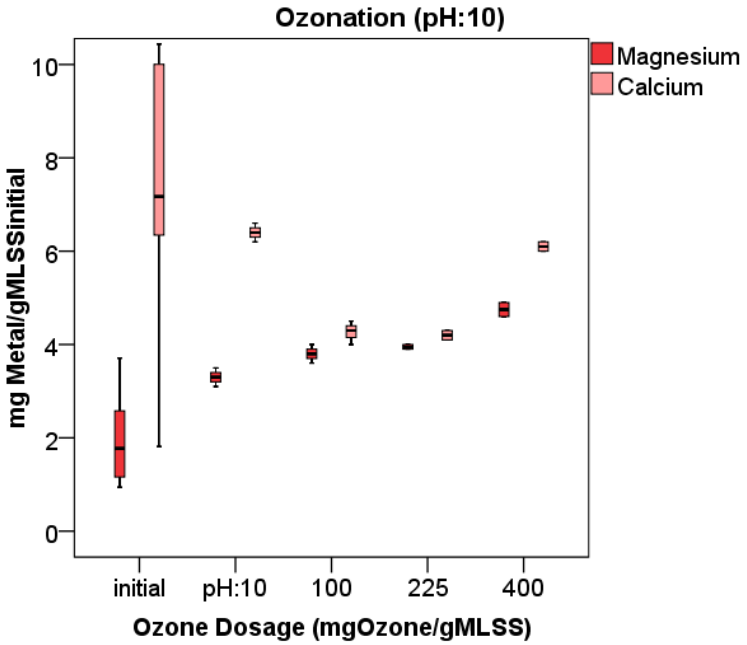


Figure 3. 34. Behaviour of magnesium and calcium during ozonation (pH:10).

Fe and Zn were also examined in ozonation studies. While both ionic and intracellular Fe concentrations showed irregular profiles during ozonation, a slight increase in Zn was also obtained as shown in Figure 3.35.

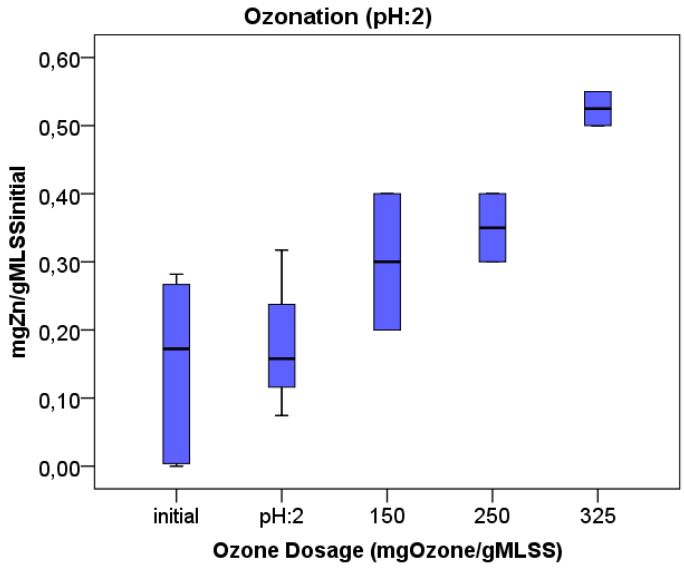


Figure 3. 35. Behaviour of zinc during ozonation.

A significant magnesium release was observed in microwave treatment of acidic pre-treated and ozonated sludge samples (Figure 3.36). Wang et al. (2016) reported the release of magnesium by microwave treatment at acidic pH conditions also. However,

in contrast to the study of Wang et al. (2016), a lesser release was also observed in microwave treatment of alkaline pre-treated and ozonated sludge.

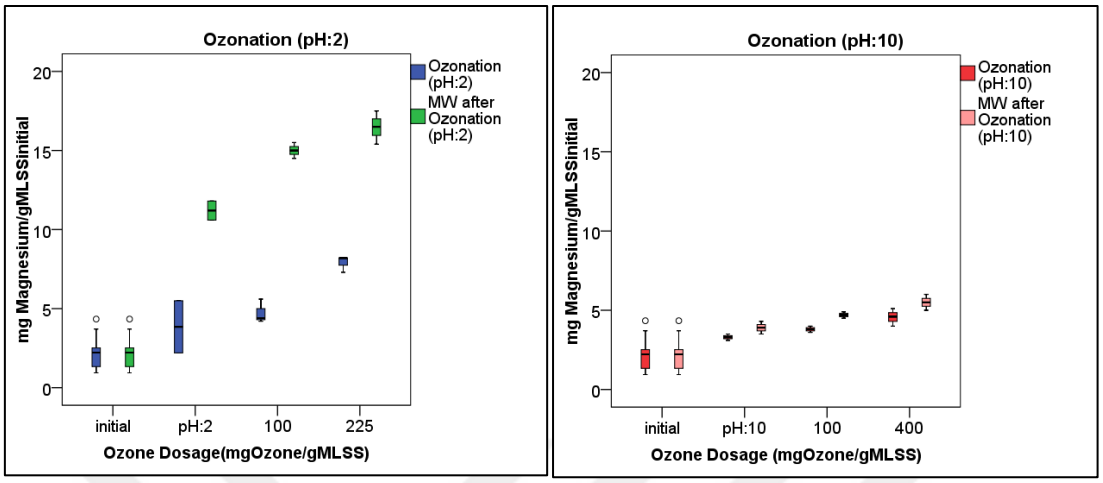


Figure 3. 36. Magnesium release with microwave treatment of ozonated sludge samples (pH:2 and pH:10)

3.6. Struvite Precipitation

Struvite precipitation is widely studied due to importance of phosphorus recovery. Lots of information about optimum conditions for precipitation can be found in the literature. Bi et al. (2014) suggested the Mg:P ratios between 1.4 – 1.8 to eliminate the precipitation of other Mg forms like $MgKPO_4$ or $Mg(OH)_2$. Higher pH levels than 9 causes precipitation of apatites and smaller MAP crystal size (Doyle and Parsons, 2002 and Rahman et al. 2014). Through the different pH levels and Mg:P:N ratios in the literature pH:8.5, pH:9.5 and pH:11, and 1.4-1.8/1/1 ratios were studied in batch tests and Mg and Ca concentrations in the precipitates were analyzed by atomic absorption spectrophotometer. pH:8.5 was chosen due to highest Mg and lowest Ca concent of precipitates for the lab scale precipitation system. Also using fine struvite particles as seed and relatively long crystallization time as two days has increased struvite precipitation efficiency.

As shown in Figure 3.37.1, brownish struvites were obtained from ozonated and microwave-ozonation mixture sludge supernatants. While 0.22 gram struvite could be obtained from per one liter ozonated sludge supernatant, up to 0.88 gram struvite could be precipitated from one liter microwave treatment-ozonation cocktail. XRD analyses

showed the existance of the struvite of the precipitates (Figure 3.37.2). Still, there is a need for a studying purification of struvite precipitates.

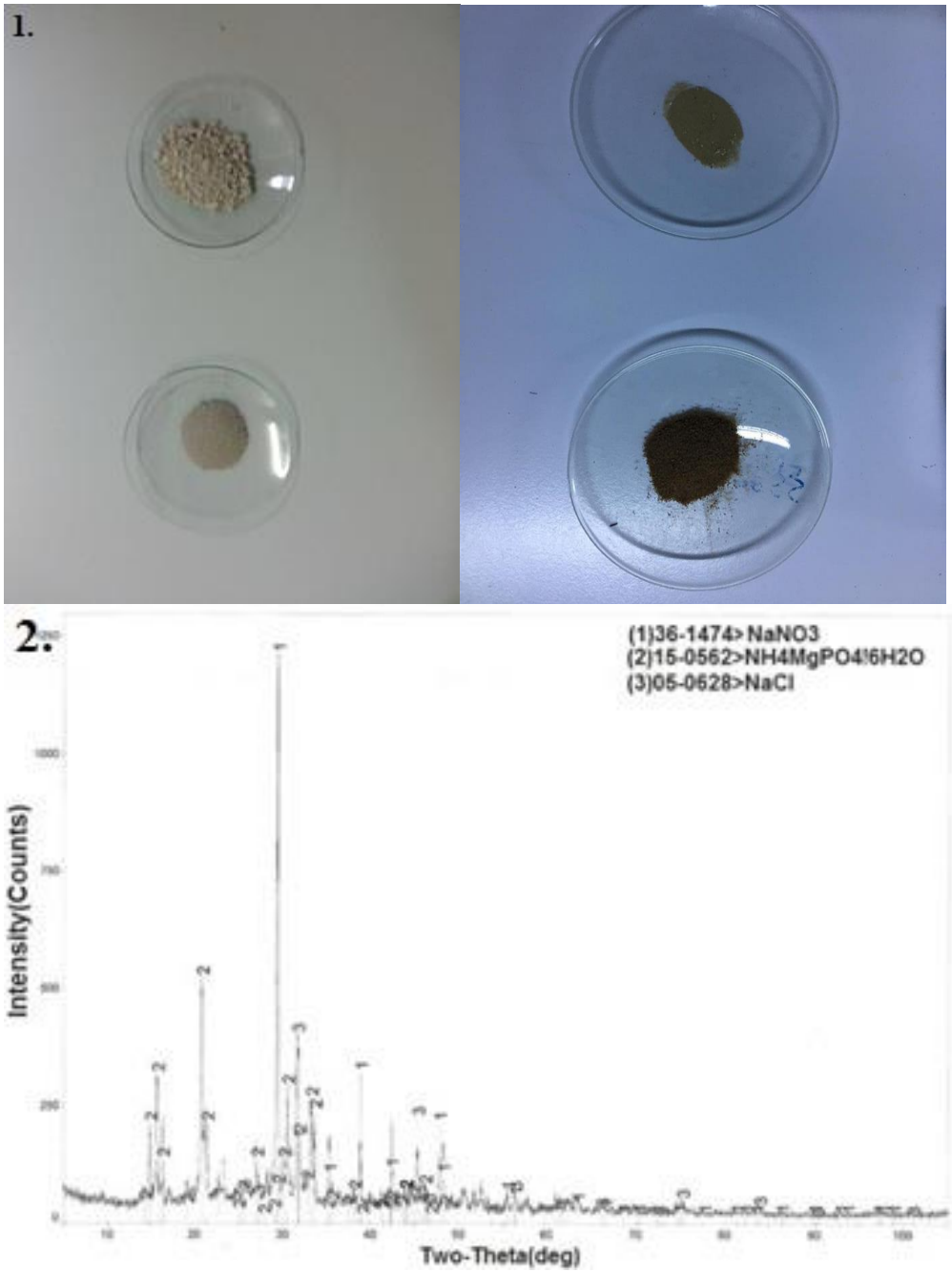


Figure 3. 37. 1. Struvite samples obtained from ozonation and microwave/ozonation experiments, **2.**XRD analysis of struvite sample obtained from ozonation.

4. CONCLUSION

Sludge disposal is one of the major components of the operating cost of a biological wastewater treatment plant and activated sludge is a rich source of phosphorus. Therefore, phosphorus recovery from waste activated sludge is a good alternative for both cost effectiveness of biological wastewater treatment and phosphorus depletion. In the scope of this study, 26 batch experiments were performed to optimize ozonation as an alternative process for phosphorus recovery. By ozonation, 20.2% COD release and 3.6 $mgPO_4$-$P/gMLSS_{initial}$ phosphorus were obtained where initial phosphorus content of sludge was 1.9 $mgPO_4$-$P/gMLSS_{initial}$. Solids reduction with 26.8% MLSS and 33.1% MLVSS solubilizations and an increase in ammonia concentration accompanied to the sludge solubilization as expected. But excessive ozonation was defined as ineffective for phosphorus release, and also caused mineralization.

Different pH pre-treatments were applied prior to ozonation in some experiments. Among the pH:2, pH:4 and pH:10 pre-treatments, pH:2 and pH:4 resulted with higher solubilizations (2.4% and 2.3% COD releases), solids reductions (8% and 7% MLSS, 5.8% and 6% MLVSS solubilizations) and phosphorus releases (3.6 and 3.9 $mgPO_4$-$P/gMLSS_{initial}$) than pH:10 pre-treatment (1.3% COD release, 3.8% MLSS solubilization, 5.3% MLVSS solubilization and 2.4 $mgPO_4$-$P/gMLSS_{initial}$). However pH:10 pre-treatment enhanced ozonation efficiency the most, probably due to the forming hydroxyl radical in the presence of high concentration of OH^-. 23.9% COD release, 35.5% MLSS solubilization, 49% MLVSS solubilization and 4.8 $mgPO_4$-$P/gMLSS_{initial}$ phosphorus concentration was obtained by 225 mgO_3/gMLSS ozone dosage after pH:10 pre-treatment, however excessive ozonation has caused to the loss of part of the released phosphorus most probably due to struvite or apatite precipitation. Acid pre-treated sludge samples needed higher ozone dosages to achieve the same phosphorus content, 4.7 $mgPO_4$-$P/gMLSS_{initial}$ phosphorus obtained by 325 and 300 mgO_3/gMLSS ozone dosages after pH:2 and pH:4 pre-treatments with 11.4 & 18.7% COD releases, 20.7 & 32% MLSS, and 20.3 & 34.1% MLVSS solubilizations. Also, both sludge samples from aeration tank and recycle line were defined as suitable for phosphorus recovery via ozonation with no significant differences due to both sludge solubilization (p:0.325) and initial PO_4-P content (p:0.246).

Microwave treatment also examined as main or post treatment for phosphorus release from waste activated sludge. Microwave treatment without any pH pre-treatment was much more effective on both sludge solubilization (25.9 % COD release, 24.5% MLSS and 29.5% MLVSS solubilizations) and phosphorus release (5.2 mgPO_4-P/g$MLSS_{initial}$) than ozonation at neutral condition. By pH pre-treatment, effect of microwave treatment enhanced significantly. Up to 27.5% COD release 31.6% MLSS & 46.2% MLVSS solubilizations, and 10.9 mgPO_4-P/g$MLSS_{initial}$ phosphorus could be obtained by acidic pre-treatment followed by microwave experiments. pH:10 pre-treatment enhanced the microwave efficiency by 26.5% COD release 24.5% MLSS and 40.9% MLVSS solubilizations, and 6.3mgPO_4-P/g$MLSS_{initial}$.

On the other hand, highest sludge solubilization and phosphorus release was observed by microwave treatment of ozonated sludge samples. 50.5% COD release accompanied by 49.5% MLSS & 58.7% MLVSS solubilizations, and 13.4 mgPO_4-P/g$MLSS_{initial}$ phosphorus content occurred after microwave treatment of ozonated and acid pre-treated sludge samples and only 15% of total phosphorus remained in particulate phase. Highest phosphorus concentration achieved by alkali pre-treatment/ozonation/microwave treatment configuration was 10.9 mgPO_4-P/g$MLSS_{initial,}$ as similar with the microwave treatment directly after pH:2 pre-treatment.

Interestingly, an increase in total COD was observed after microwave treatment of ozonated sludge samples probably due to release of materials such as proteins polysaccharides which could not be detected by COD test at first.

Precipitation of released phosphorus as struvite was also examined in the scope of this study. Optimum pH for struvite precipitation were determined as 8.5. and using fine struvite particles as seed for crystallization enhanced the recovery efficiency.

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Scientific Works

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Coşgun S. and Semerci N.” Phosphorus Recovery from Waste Activated Sludge: Comparison of Microwave Treatment and Ozonation in Acidic Environment”. International Conference on Recycling and Reuse, Istanbul, Turkey 2016 (Oral Presentation).

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