MARMARA UNIVERSITY

INSTITUTE FOR GRADUATE STUDIES

IN PURE AND APPLIED SCIENCES

ANAEROBIC DIGESTION OF WASTE ACTIVATED SLUDGE: INFLUENCE OF CO-SUBSTRATE IN THE BIOLEACHING OF HEAVY METALS

RAHIM MOLAEY

Ph.D. THESIS

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Rahim MOLAEY, Ph.D. student at Marmara University, Institute for Graduate Studies in Pure and Applied Sciences, defended his thesis entitled "Anaerobic Digestion of Waste Activated Sludge: Influence of Co-Substrate in the Bioleaching of Heavy Metals", on October 5th, 2021 and has been found to be satisfactory by the jury members.

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Marmara University Institute for Graduate Studies in Pure and Applied Sciences Executive Committee approves that Rahim MOLAEY be granted the degree of Doctor of Philosophy in Department of Environmental Engineering, Environmental Engineering Program on ……. (Resolution no: …….).

Director of the Institute
Prof. Dr.

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September, 2021 **Rahim MOLAEY**

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ÖZET

ATIK AKTİF ÇAMURUN ANAEROBİK ÇÜRÜMESİ: EŞ-SUBSTRAT'ın AĞIR METAL BİYO-SIZMASI ÜZERİNE ETKİSİ

Nüfus artışı ve daha katı mevzuatların gereği olarak kapsamlı atık su arıtma sistemlerine olan ihtiyaç biyolojik atıksu arıtma tesislerinde fazla arıtma çamuru üretilmesine neden olmaktadır. Bu bağlamda, stabil biyolojik arıtma çamurlarının tarım arazilerine uzun süre uygulanması sonucu topraktaki ağır metal miktarının artma olasılığı endişe vericidir. Amacı bu soruna çözüm üretmek olan bu tez çalışmasında, biyolojik ve fiziko-kimyasal arıtma proseslerinin kombinasyonundan oluşan hibrit bir teknoloji geliştirilmiş ve potansiyel toksik ağır metallerin stabil biyolojik arıtma çamurundan uzaklaştırılmasına çalışılmıştır. Bu amaçla, metallerin anaerobik olarak biyo-sızdırılması işlemini test etmek için çamur yaşı 4 gün ve 16 gün olan asidojenik ve metanojenik iki ayrı reaktör işletilmiştir. Asidojenik reaktörlerde, KOİ eş değeri olarak 67,200 mg/L toplam uçucu yağ asidi (UYA) üretimi sonucunda arıtma çamurunun katı fraksiyonundan %26 Ni giderilmiştir. Kromun çözünmüş organik maddeler ile kompleks oluşturduğu düşünülmekte olup nötr pH'da toplam Cr'un sadece %24'ü sıvı fraksiyona sızmıştır. Çalışmanın son bölümünde, fermente çamura havalandırma (0,875 L/L-fermente ürün.dk) ile desteklenen ultrases (9kJ/g katı madde) uygulanmış ve en az %83 Ni, %82 Cu ve %80 Zn biyo-sızma verimi elde edilmiştir. Bu sonuçlar, önerilen hibrit sistemin, CH4 formunda eşzamanlı enerji geri kazanımı ile stabilize biyokatı ürettiğini ortaya koymuştur. Bu sonuçlar, önerilen hibrit sistemin biyolojik atıksu arıtma çamurlarından anaerobik çürütme ile eş zamanlı metan formunda enerji geri kazanımı ve ağır metal miktarı ciddi şekilde azaltılmış stabil çamur üretiminin başarıyla gerçekleştirilebileceğini göstermiştir.

Anahtar Kelimeler: Anaerobik Biyo-sızma, Fermente ürün, Ağır Metal, Atık Çamuru, Uçucu Yağ Asidi, Nihai Arıtma

ABSTRACT

ANAEROBIC DIGESTION OF WASTE ACTIVATED SLUDGE: INFLUENCE OF CO-SUBSTRATE IN THE BIOLEACHING OF HEAVY METALS

Biological wastewater treatment, generates considerable amounts of sludge as a by-product; that is mainly correlated to the growth of population, and extensive wastewater purification as required by stricter legislations. In this regard, recurring application of stabilized sewage sludge to agricultural lands, is of great concern due to possibility of increasing metal content of soil. To overcome this problem, in the current thesis study, a hybrid technology; combination of biological, and physical-chemical treatment processes, was developed to remove potentially toxic metals from stabilized sewage sludge. In this respect, anaerobic bioleaching process was carried out in anaerobic acidogenic fermenters with sludge retention time (SRT) of 4 days and anaerobic methanogenic reactors with SRT of 16 days. Accumulation of volatile fatty acids (VFAs) up to 67,200 mg VFA-COD eq/L in acidogenic reactors along with 26% removal of Ni from the solid state of sewage sludge was observed. Presumably due to complexation of Cr with dissolved organic matters (DOM), 24% of the total concentration of Cr was leached into the liquid fraction at neutral pH. In the last part of the study, application of air-assisted ultrasonication technique with specific energy input greater than 9 kJ/g total solid (TS) through ultrasonication and supplying air with constant flow rate of 0.875 L of air/ (L of digestate·min), resulted in leaching of more than 83% of Ni, 82% of Cu and 80% of Zn. These results revealed that the proposed hybrid system, generates stabilized biosolid with simultaneous energy recovery in the form of CH_4.

Keywords: Anaerobic Bioleaching, Digestate, Potentially Toxic Metals, Sewage Sludge; Volatile Fatty Acids, Post-treatment

CLAIM FOR ORIGINALITY

Sludge management is the main issue of water quality engineering. The future concerns regarding the water usage, beneficial reuse of energy and conservation, recycling and environmental protection will demand more of sewage sludge. Therefore, biosolid production from sewage sludge is the main component of water and sanitation engineers. However, mainly depending on the composition of the wastewater, sewage sludge typically contains a diverse range of recalcitrant and potential hazardous contaminants such as potentially toxic metals (HM). Presence of HMs are serious environmental concerns associated with waste activated sludge (WAS). They have been released in sewerage lines and have been frequently found in wastewater treatment plants (WWTP). Their availability in WAS, has aroused a great deal of public concerns due to their persistence and ecotoxicological effects on human health, terrestrial, and aquatic life. In this respect, urban waste water treatment directive (UWWTD) in Europe made the regulations stricter for further usage of sludge as soil conditioner and/or fertilizer. Therefore, regional and national regulations need to dictate proper treatment of wastewater and sludge before resource recovery and disposal. Conventional wastewater treatment processes have minor influence on the HM leaching from the solid state of WAS. On the other hand, using advanced wastewater treatment processes, for HMs removal are quite expensive. In this respect, for the first time, the results of this thesis provided a novel hybrid technique, i.e., anaerobic digestion (AD) + air assisted ultrasonication as a post-treatment process, for the leaching of HM from the sludge. For the first time in this technique, air-assisted ultrasonication (US) has been introduced as a completing stage after the bioleaching processes to decrease the total treatment time and to rule out the issue of high energy consumption of using only ultrasound. In this process, cavitation was highly produced by bubbles generated by low frequency ultrasonication of the liquid phase. Aeration accompanied the ultrasonic irradiation, further strengthened degradation efficiency by disrupting the structure of organic flocs; and therefore, high HM leaching efficiency from the solid state of sludge was obtained. Given the worldwide increasing interest in sustainable sludge treatment processes, circular bioeconomy and environmental protection in general, it is expected that the obtained results in this research work, will be quickly picked up by the scientific community and valorized by more practically-oriented research groups or companies.

SYMBOLS

%	: Percent
C	: Carbon
Ca	: Calcium
Cd	: Cadmium
CH_4	: Methane
cm	: Centimeter
CO_3^{2+}	: Carbonate
CO_2	: Carbon dioxide
Cr	: Chromium
Cu	: Copper
d	: Day
g	: Gram
h	: Hour
H_2	: Hydrogen
Hg	: Mercury
K	: Potassium
kg	: Kilogram
kW-h	: Kilo watt hour
L	: Liter
M	: Million
m^3	: Cubic meter

mg	: Milligram
Mg^{2+}	: Magnesium
min	: Minute
MJ	: Mega Joule
N	: Nitrogen
Ni	: Nickel
NH_3	: Ammonia
NH_4^+	: Ammonium
^{o}	: Degree
^oC	: Degree Celsius
OH^-	: Hydroxide ion
P	: Phosphorus
Pb	: Lead
pH	: Hydrogen ion activity
rpm	: Revolution per minute
s	: Second
S	: Sulfur
W	: Watt
Zn	: Zinc

ABBREVIATIONS

AD	: Anaerobic digestion
AF	: Anaerobic fermentation
AOS	: Adsorbed organic substances
CB	: Cell biomass
COD	: Chemical oxygen demand
DOC	: Dissolved organic carbon
DOM	: Dissolved organic matter
DS	: Dry solid
EDTA	: Ethylene di-amine tetra acetic acid
EOS	: Extracellular organic substances
EPS	: Extracellular polymeric substances
EU	: European Union
HMs	: Potentially toxic metals
HIV	: Human Immunodeficiency Virus
NTA	: Nitrilo tri-acetic acid
RCF	: Relative centrifugal force
TAN	: Total ammonia nitrogen
TKN	: Total Kjeldahl nitrogen
TS	: Total solids
US	: United States
VFA	: Volatile fatty acids

VS	: Volatile solids
WAS	: Waste activated sludge
WWTP	: Wastewater treatment plant

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1. INTRODUCTION

Increasing urbanization and industrialization resulted in considerable increase in the volume of wastewater worldwide. Origins of wastewater can be listed as domestic, industrial, and municipal wastewater. Domestic wastewater originates from households, industrial wastewater is generated through industrial activities, and municipal wastewater is generated through both domestic and industrial activities. Wastewater is normally gathered through sewage pipes and then treated through the combination of physical and biological treatment processes. After separation of liquid and solid parts, water can be introduced back into the environment and solid can be applied to land as an organic fertilizer. The treatment involves a step-wise processes composed of primary, secondary, and tertiary treatment. Primary treatment is carried out using basic screening and sedimentation processes to remove suspended solids and to decrease its biodegradable chemical oxygen demand (bCOD) up to 30%. In the secondary treatment processes, approximately 90% bCOD remaining after the primary treatment, can be biologically degraded. Tertiary treatment sometimes called advanced treatment, which includes purification processes applied beyond the former steps and may involve more complex technology such as reverse osmosis, ion-exchange to further eliminate nutrients or pollutants (Malik et al., 2015).

In biological wastewater treatment, the term sludge recalls two basic forms, i.e., sludge and secondary sludge which might be called as activated sludge in the case of using activated sludge processes (Demirbas et al., 2017). Residue from primary sedimentation tank and the settled microorganisms generated in biological processes and after the secondary sedimentation tanks are collectively named as sewage sludge, that is the major portion of the wastewater treatment byproducts. In order to put sewage sludge into value or to manage for its safe disposal, sewage sludge needs to undergo thickening, stabilization, conditioning and dewatering processes (Fernandez et al., 2020). Therefore, according to the applied treatment processes, the generated sludge contains 2 – 30% dry solid (DS) and needs to be transferred from residential sites for distribution in landfills, incineration facilities or recycle in land application (Peccia and Westerhoff, 2015). On the other hand, sewage sludge can be used as an asset greater than its current use as a soil conditioner. High value-added materials such as nutrients and energy embedded in it can be recovered with the help of anaerobic biodegradation processes, are the examples of extractable assets from sewage sludge (Peccia and Westerhoff, 2015). In this context, anaerobic

digestion (AD) is the most promising method for stabilization and hence sanitation of sludge. In these processes, microorganisms eradicate biodegradable portion of sewage sludge and generate biogas mainly composed of CH_4 and CO_2, while significantly enhances fertilizing properties of residues named digestate (Möller, 2015). Assuming in each year 1 million dry tons of sewage sludge is generated in Turkey (Abusoglu et al., 2013) with nitrogen (N) and phosphorus (P) content of 3.4 and 2.3%, respectively (Peccia and Westerhoff, 2015), then the amount of N and P contents of generated sludge is accounted to be in the order of 34,000 tons N/year and 23,000 tons P/year. Moreover, sewage sludge representing embedded energy in the range of 15 to 20 MJ/kg DS of sludge. By practicing energy extraction from sewage sludge in the AD processes, more than 5,555,000,000 kWh, of energy can be recovered.

1.1. Generation of Sewage Sludge and Current Management Processes

Generation and safe disposal of sewage sludge, are considered as the main goal of water and sanitation engineers. The disposal of this by-products is considered as one of the most complex environmental issues. This is because the generated sludge in the WWTP is in the form of dilute suspension, and may involve only 0.25% solids, based on the processes applied in the WWTPs (Werther and Ogada, 1999). With rapid increase in population and urbanization, centralized collection and treatment of wastewater, generated large amount of sewage sludge in the wastewater treatment processes. This large amount of generation, is an inescapable drawback of sewage sludge processes. Daily amounts based on DS has been reported to be within the range of 60 to 90 g DS per capita, which is equivalent to more than 13 million tons of dried sludge per annum in the European Union (EU) countries (Bratina et al., 2016; Molaey et al., 2021a). Current generation of sewage sludge in Turkey is above 1 million dry tons of excess sludge per annum (Salihoglu et al., 2007). The first WWTP in Turkey was built in 1982 with total capacity of 751,000 m^3 per year. Within 12 years and by the end of 1994 the total number of WWTPs increased to 45 with total capacity of 602 Mm^3 per annum. Most of the generated wastewater was treated biologically in 41 WWTPs that accounted for 37.35% of the total treated water (Abusoglu et al., 2013). Within 16 years starting from 1994, construction of WWTPs significantly increased and reached to 326 WWTPs with total capacity of 5,293 Mm^3 per annum by the end of 2010. Most of the WWTPs have primary and secondary treatment processes which accounts for 91% of the total capacity.

Total of 52 provinces in Turkey had urban WWTPs and almost 73% of the total civilians being served by the end of 2010 (Abusoglu et al., 2013).

The origin and nature of sewage sludge, may cause a hygiene related health hazards during the treatment processes. Moreover, toxic organic matters, macro and micro nutrients, organic micro pollutants, pathogens and HMs are serious environmental concerns associated with sewage sludge (Leng et al., 2016). From one side, strict regulations make the land application of sewage sludge less favorable due to concerns regarding soil contamination and human health risks (Peng et al., 2011). On the other hand, sewage sludge is carbonaceous in origin containing carbon and nutrients that has the potential to be utilized as a renewable resource rather than a waste (Dong et al., 2013). Current management systems of sewage sludge are multitudinous and sludge treatment usually composed of thickening, dewatering, stabilization and drying with the help of various physical, chemical and biological processes. The disposal methods such as sanitary landfill, incineration and landfill application have also been applied (Yang et al., 2015). With rising the demands in environmentally sustainable processes, current management system limits the resource utilization of sludge and urgent need for environmental benign alternatives are necessary. Hence, periodic monitoring of certain HMs and pathogen in the treated sludge is required.

1.2. Composition of Sewage Sludge

To find the appropriate method for disposal of sewage sludge and to ensure sustainability of the applied method, composition of sewage sludge is of great importance. Sewage sludge mainly contains non-toxic organic matter with high energy potential that can be used as a resource for energy recovery (Zhen et al., 2015). Excluding non-toxic organic compounds, like any other urban wastes, sewage sludge may contain various type of pathogens that are infectious for plants and animals (Leng et al., 2016). Chemical composition of sewage sludge shows locational and seasonal variations. Therefore, it is necessary to periodically monitor physical and chemical characteristics of sewage sludge upon its disposal. As mentioned in the Introduction (Section 1), sewage is the by-product of domestic, industrial, and commercial activities or in general what excreted from the society. It is the heterogeneous mixture of feces, urine, vomitus, etc. and composed of organic matters, macro and micronutrients, micropollutants, microorganisms and approximately, 43 various types of human viruses, e.g., Adenovirus, Corona virus and Human Immunodeficiency

Virus (HIV), etc. (Bibby and Peccia, 2013). Other harmful substances, e.g., detergents, various salts and pesticides may also present in sewage sludge due to municipal and industrial discharges into the sewer line (Singh and Agrawal, 2008). Persistent organic chemicals, e.g., retardants, various types of antibiotics, narcotics and metabolites from these compounds are commonly existed in sewage sludge (Chari and Halden, 2012). Sewage sludge may also contain potentially toxic metals (HM), e.g., Cr, Cu, Ni and Zn as a consequence of municipal and industrial discharges to the sewer systems (Singh and Agrawal, 2008). Based on the properties of sludge structure, organic matter in sludge can be categorized into cell bacteria and extracellular organic substances (EOS). Cell bacteria is the combination of cell membranes and intracellular organic substances. EOS involved extracellular polymeric substances (EPS) and adsorbed organic substances (AOS). It has been found that EOS content of sewage sludge is more than 80% of the volatile solid (VS) (Xu et al., 2018), which indicates high concentration of EOS in sewage sludge. With increasing the sludge age, the EOS content of the sewage sludge will increase significantly (Brown and Lester, 1982). This can be described by the presence of more mature microbial communities that has low growth rate and high extracellular biopolymer storage (Figure 1.1). Moreover, as the sludge gets more mature, its tendency for adsorbing organic substances from wastewater also increases. Macronutrients in the sewage sludge are considered as a source of plant nutrients and the organic matter content of sewage sludge enhances the conditioning properties of the soil (Logan and Harrison, 1995). Recycling of sewage sludge and application on land can supply major plant nutrients, e.g., N, P and potassium (K) presented in sludge (Mark Gerard Healy, Racjel M Clarke, Dara Peyton, 2012). Major portion of N in sewage sludge is within the organic fraction originated from cellular organic compounds such as proteins, nucleic acid, and amines. Sewage sludge also contains inorganic nitrogen in the form of ammonium nitrogen (NH_4-N) and small amount of nitrate-N (NO_3-N) based on the treatment processes used (Sommers, 1977). Secondary nutrients, e.g., calcium (Ca), magnesium (Mg), sulfur (S), and many other trace metals such as iron (Fe), copper (Cu), nickel (Ni), chromium (Cr), and zinc (Zn) may also exist in sewage sludge (Sommers, 1977). For commercial crops, application of sewage sludge as a fertilizer to supply N fertility, provides substantial amount of P but suboptimal levels of K to the crop. Ammonium N being already available whereas the organic N become available to crops over several years. The majority of P and K in sewage sludge presents in inorganic fractions and 50% of P and 100% of

K in sewage sludge are accessible to be up taken by plants (Mark Gerard Healy, Racjel M Clarke, Dara Peyton, 2012; Singh and Agrawal, 2008; Smith and Durham, 2002).

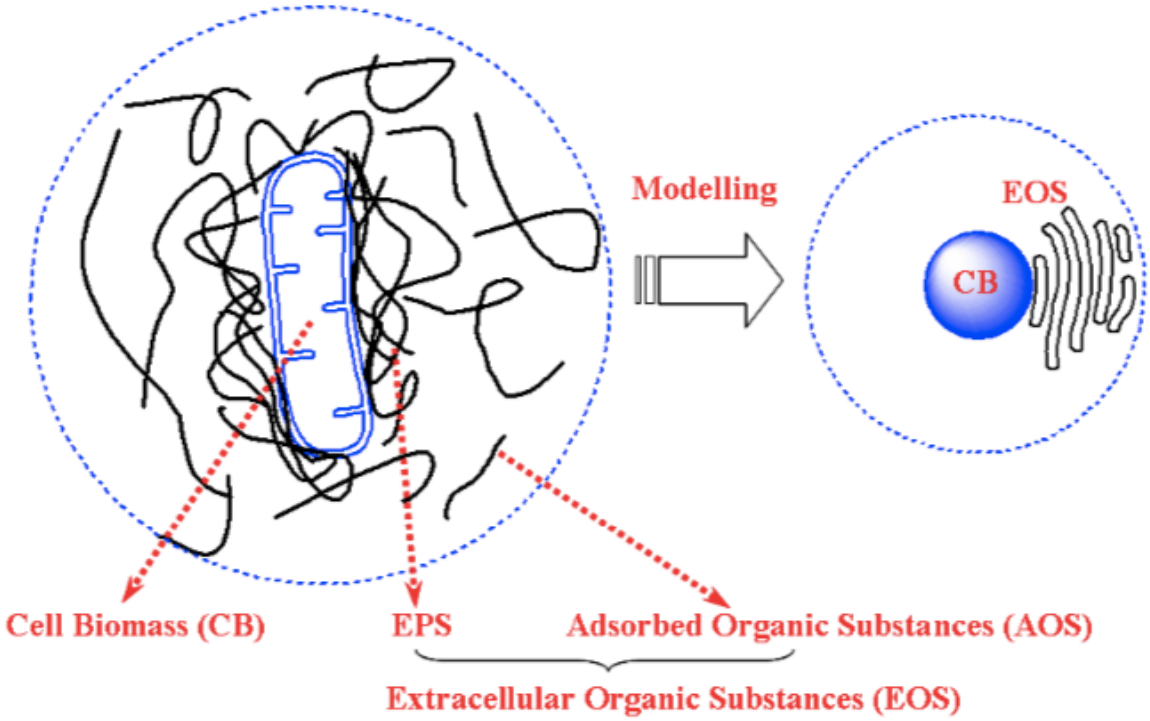

Figure 1.1. Representation of sewage sludge organic substances

(Xu et al., 2018)

1.3. Metal Content of the Generated Sewage Sludge

Despite the continuous innovations in wastewater treatment engineering, production of sewage sludge at WWTPs cannot be avoided. Final disposal of this waste is a matter of great concern since HMs accumulate in the sewage sludge during wastewater treatment processes. Recent investigations seek to encourage the application of sewage sludge in agriculture and manage its use in a safe way to decrease the damaging effects of sewage sludge in the environment (Peccia and Westerhoff, 2015). Combination of road run off, domestic and industrial wastewater into municipal discharge systems produce sewage sludge polluted with HMs (Bezzina et al., 2019). HMs such as Cr, Cu, Ni and Zn in sewage sludge are difficult to be dealt with because of their different properties under various conditions. In the periodic table of elements these metals are

categorized as transitional elements and can form compounds with different oxidation states, resulted from their complex valence shell. They are non-biodegradable and their concentrations are generally high in sludge that limits the sludge disposal for agricultural purposes (Xu et al., 2018).

Allowable concentrations of HMs in soil for EU' member states and in Turkey are given Table1.1.

Table 1. 1. Limit values for concentration of potentially toxic metals in soil for EU and Turkey

Metal (mg/kg)	EU [a]	Turkey [b]
Cr	-	-
Cu	50 - 140	4.17
Ni	30 - 75	16.67
Zn	150 - 300	50
Cd	1 - 3	6.67
Hg	1 – 1.5	5
Pb	50 - 300	4.67

[a](Inglezakis et al., 2014); [b](Yaman and Olhan, 2012)

As a consequence of land application of stabilized sewage sludge, toxic metals may be released back into the water systems or leach into the environment at a later time. Based on the directive 86/278/EEC national legislation of EU, which has been established in the member states, application of sludge for agricultural purposes is prevented when the concentrations of these elements are more than the permissible limit values (Inglezakis et al., 2014). Concentrations of HMs in sewage sludge are highly influenced by the characteristics of the incoming wastewater and types of the treatment systems in the WWTPs and hence they show significant local variations.

1.1. Application of Anaerobic Digestion Processes for Sewage Sludge Treatment

Sewage sludge is the byproduct of WWTPs, generated from different units during physical, chemical, and biological processes that involve sedimentation tanks, biological tanks, thickener, centrifuge, etc. as explained in Section 1.2. Increasing concern regarding disposal of generated sludge, necessitates the research community to treat the sludge in effective ways. Apart from huge amounts of wastewater generation, the processes of reuse and disposal are the most common and complex issues that environmental and sanitation engineers are facing. The complexity comprises a variety of processes that occur at vastly different rates. In this regard, inter-related process such as mass transfer, hydraulics, and chemical reactions all occur simultaneously that increase the complexity of the processes. Therefore, selection of the efficient process includes number of possibilities which are inter-related. In this context, completion of variety of objectives and multiple criteria, increases the complexity of the treatment processes (Garrido-Baserba et al., 2015). Environmental and economic factors are also needed to be considered along with technical issues in the treatment processes (Garrido-Baserba et al., 2015). The cost of sludge management, plays a key role in WWTP and the solid handling and treatment processes account up to 50% of the total treatment facility's costs (Garrido-Baserba et al., 2015). However, it should be noted that embedded energy in sludge that can be recovered in the AD processes, is 10 times higher than the energy required to treat it. Therefore, new perspectives have been considered to recover energy from sludge, which can be directly used in wastewater treatment facilities and reduce the energy dependence on conventional electricity. With regard to sustainability, sludge treatment technologies need to be designed as solutions not exempted of negative impacts. Therefore, combination of direct impacts that are associated with sludge treatment and indirect impacts that are accompanied to the inputs (facilities and energy use) and outputs (emissions and waste generations), has to be determined during the decision-making processes for selection of the sludge treatment technology.

Despite decades of attempts to utilize sewage sludge in terms of resource recovery, the term used as sludge is often discerned as an unpleasant left over of wastewater production and brings number of questions and problems related to pathogens in a wide range and chemicals presented in the wastewater. To solve these questions and problems, management of sewage sludge has been carried out using three basic methods, e.g., land application, incineration, and landfilling. Combustion is the most widely used thermal treatment process for disposal and sludge energetic

valorization. Wet or dry sludge combustion, with TS content of 41% to 65%, can be used in the combustion reactors (Samolada and Zabaniotou, 2014). However, with stringent regulations concerning environmental issues, sewage sludge incineration has been recognized as the major source of inorganic and organic hazardous air pollutants. Inorganic pollutants, e.g., Cd and Hg are transferred with volatile organic matters and released into the environment (Fullana et al., 2004). Organic pollutants may also be generated with sludge incineration and can be found in the incinerator stacks. Based on their chemistry and hazardous properties, pollutants released after incineration of sewage sludge they could be categorized in four different groups: (a) partially oxidized organic matters, e.g., aldehydes and phenol; (b) aromatic compounds, e.g., benzene and toluene; (c) chlorinated hydrocarbons, e.g., chloromethane; and (d) dioxin compounds (Fullana et al., 2004). As mentioned in the introduction part (Section 1), sewage sludge is a nutrient rich biowaste that contains considerable amounts of C, N and P (Formentini et al., 2017). Therefore, it can cause eutrophication if discharged without treatment to inland or coastal waters. Moreover, uncontrolled dumping and land application of sewage sludge can increase contaminant levels of soil by releasing HMs (Jung and Lovitt, 2011). Alternative to all these sludge disposal methods, generated sludge can be recycled to high value-added materials if HMs and pathogens in the sludge can be decreased with biological or chemical treatments. Therefore, the economical and environmentally sustainable method might be applied to extract nutrients and embedded energy in sewage sludge. The cost of these assets is estimated to be around $ 550 per ton. However, $300-800 per ton is the estimated cost for sludge land application (Peccia and Westerhoff, 2015). As mentioned above, sewage sludge contains considerable amounts of energy that exceeds the energy needed for its treatment (Garrido-Baserba et al., 2015). Therefore, sewage sludge treatment by applying AD processes and recovery of the embedded energy in term of biogas, WWTPs can be considered as energy producers. These benefits can be achieved by stabilization of sewage sludge using AD processes, and recover the energy associated with the production of nutrient rich digestate.

The amount of treated wastewater, generated sludge, produced biogas, and the potential of the electricity production of the 326 WWTPs located in the different geographic locations of Turkey are shown in Figure 1.2. Biogas production potential of existed WWTPs in Turkey has been reported to be higher than 200 Mm^3 per year. If the total estimated biogas production potential can

be directed to the power generation, produced electricity from sewage sludge in Turkey would be elevated to 530 GWh, that meet 1% of the total energy demand in each year for the country.



Figure 1.2. Generated sludge, biogas production and electricity generation of WWTPs in Turkey (Abusoglu et al., 2013)

It has been reported that the annual agricultural use rate of sewage sludge is about 10%. Legislations that manage the use of sewage sludge for agricultural purposes in Turkey has given much attention to the soil pollution, whereas less consideration has given for sludge valorization with compare to the EU member states (Ministry of Environment and Urbanisation, 2016).

1.4. Biochemical Processes of the Anaerobic Digestion

AD is a biochemical process that transform organic content of wide variety of wastes, e.g., sewage and industrial sludge and energy crops into (1) biogas mainly containing CH_4 and CO_2; (2) digestate, which can be used as a soil conditioner; and (3) supernatant, which can be used as an organic fertilizer (Uçkun Kiran et al., 2016). AD has been considered as the most energy-efficient and environmentally sustainable technology for energy production.

Despite many advantages of AD, the process has disadvantages such as complexity, operational instability, high sensitivity at various environmental conditions, long start up periods and retention time (Appels et al., 2008). These biological processes have been considered a very complicated process. The complexity of the process can be arranged in to three main categories, e.g., biological, operational, and chemical and each category may contain several aspects. Complexation of the process might be correlated to inherent interdependency of the biological, operational, and chemical processes involved during the digestion process (Amani et al., 2010).

Different types of microorganisms with specific properties cooperate in the AD process. The quantities of microorganisms in the anaerobic reactor is predicted to be higher than 10^{16} cells/mL (Amani et al., 2010). Microorganisms such as saccharolytic microorganisms, proteolytic microorganisms, lipolytic microorganisms and methanogens that cooperate in four different steps such as hydrolysis, acidogenesis, acetogenesis and methanogenesis (Amani et al., 2010). In the first step of the fermentation processes, which is called hydrolysis, bio-wastes are transformed to soluble organic matters that can be further converted to monomers. In hydrolysis, several extracellular enzymes, e.g., cellulases, proteases, and lipases, are cooperating to catalyze complex organic molecules, and hence this step is known as the rate limiting step during the whole digestion processes (Xu et al., 2018). Presented microorganisms in the fermentation stage, have relatively high growth rate and can tolerate extreme conditions such as low pH, thermophilic conditions and shock loadings at high organic loading rates (OLR) (Ahring et al., 2001). Anaerobic microorganisms that are responsible of generation of acetate, H_2, formate and CO_2 from the degradation of short chain fatty acids are called acetogenic bacteria (Wong et al., 2009). Afterwards, microorganisms in the last stage are methanogens and they initiate methanogenesis. Methanogens belong to the Archaea domain and their cooperation with acetogenic microorganisms

is of great importance to the performance of the whole digestion processes. In typical AD processes 70% of CH_4 produced by acetate route. CH_4 production can shift to syntrophic acetate oxidation (SAO) route based on operational parameters and feedstock. In the mentioned route separate group of microorganisms transform the acetate to H_2 and CO_2 and finally H_2 converted to CH_4 by hydrogenotrophic methanogens (Molaey et al., 2018). H_2 concentration needs to be kept to low level to facilitate thermodynamically favorable condition for the hydrogen forming acetogens to transform the organic acids into acetate, H_2 and CO_2. For this reason, H_2 is constantly consumed from the system and transformed to CH_4. By this way, H_2 concentration kept at low level that provides the equilibrium between acetogens and methanogens which is commonly named as interspecies hydrogen transfer (Wong et al., 2009). The disruption of this process or inhibition of methanogens can affect the whole digestion processes.

1.5. Co-digestion of Sewage Sludge with Carbon Rich Organic Waste

As mentioned previously (Section 1.1), despite significant energy consumption, current research estimations predict the availability of the considerable amount of energy in the sewage sludge than is consumed in the WWTPs. This indicates that if energy consumption declines and energy recovery enhances, it is possible for WWTPs to be energy positive (Dai et al., 2019). Among different types of treatments, AD of sewage sludge is favored over the others due to advantages of less capital cost and less maintenance charges (Stroot et al., 2001). On the other hand, complex structure of EPS in sewage sludge makes it difficult to be hydrolyzed and digested. The biodegradable portion of these substances is only 30 to 50% of VS, even at long sludge retention times (SRT) of 20 to 30 days in the reactor. As a results of low volatile solids contents of the sludge, low CH_4 yield is produced during the digestion process (Appels et al., 2008). To increase the hydrolysis rate and hence subsequent CH_4 yield, different pretreatment processes such as thermal, chemical mechanical, biological or combination of these processes have been applied (Houtmeyers et al., 2014; Kim et al., 2010; Zhen et al., 2017). Although pretreatment enhances CH_4 yield, high energy demand and capital costs are the major drawbacks of these technologies. Manipulating the composition of the wastes to manage the nutrient deficiency and balance the C/N might be a good solution. Sewage sludge is the most common waste in the world, the co-digestion is also viable all the times. During co-digestion, simultaneous degradation of homogeneous mixture of two or more biomass occurs. These processes motivate distinct organization in finding

solutions to manage different types of wastes produced in the society (Rao and Baral, 2011). Co-digestion of sewage sludge with other organic wastes such as greenery, fruit and vegetable wastes can increase the solid contents and nutritional balance during the digestion process (Rao and Baral, 2011). Co-digestion of sewage sludge not only enhances CH_4 yield but also increases the stability of the process and solids degradation efficiency as various kinds of wastes are being mixed (Rao and Baral, 2011).

Greenery from public spaces such as grass is a potential source of biomass for anaerobic digesters. In recent years, although uses of grass wastes for forage production is diminishing, the demand for biomass energy is increasing (Hidaka et al., 2013). Grass waste is a suitable substrate in many ways for AD process, not only because of its high carbon content but also its abundance in the environment (Rodriguez et al., 2017; Zhen et al., 2015). In anaerobic co-digestion of WAS with greenery wastes, C/N ratio can be well balanced and ammonia inhibition or acidification of the process will be eliminated (Hidaka et al., 2013). Concurrently, using grass as a co-substrate in AD process may efficiently decrease the improper disposal such as burning whilst preventing green-house gas emission (Zhen et al., 2015).

1.6. Bioleaching of Potentially Toxic Metals

Environmental pollution is incurred from both natural occurrence and anthropogenic activities, e.g., industrial manufacturing, agricultural activities, organic disposal, etc. Particularly, land application of metal-laden sewage sludge has been considered as a contamination source. Toxic metals may be up-taken by plants and accumulate in the plant tissues, thereby contaminate the terrestrial and aquatic ecosystems (Selling et al., 2008). In recent years, number of research attempts have been done to decontaminate sludge from metals prior to its land application. Different types of chemicals, e.g., $Fe_2(SO_4)_3$ and $FeCl_3$, chelating agents, e.g., ethylene di-amine tetra acetic acid (EDTA) and nitrilotriacetic acid (NTA), organic and inorganic acids have been used to extract metals from sewage sludge (Pathak et al., 2009). Consumption of large amounts of chemicals, number of operational problems, and high operation costs are the major drawbacks of these technologies (Pathak et al., 2009). Therefore, dissolution of metals from wastes, needs to be directed to the microbial based processes. These processes are the combination of two major areas; chemistry and microbiology, that were put into another subject area named biohydrometallurgy (Singh and Cameotra, 2015). In the view of current researches, biohydrometallurgy is one of the

most important solutions for treatment of various types of wastes and it has attracted attention as the strict antipollution regulations are enforced.

When microorganisms interact with metals of a complex solid and leached them, the process is called bioleaching (Pathak et al., 2017). In this process, required energy is provided as the metal form is changed due to redox reaction catalyzed by microorganisms. Therefore, catalytic influence resulted from the metabolic activity of the microbial communities, can play important roles in the bioleaching of HMs (Camargo et al., 2016).

1.6.1. Aerobic Bioleaching Processes

In the aerobic bioleaching processes, responsible microorganisms are aerobic chemolithoautotrophs with wide variety of nutritional needs, most of them might be provided by aerating the sludge slurry. Oxygen (O_2) is provided through aeration, while sludge provides required organic matters, mineral nutrients and to some extent trace elements. Microbial activities may influence the fate of metal ions in a direct and indirect ways (Figure 1.3). In the direct way, microorganisms contact to metal via EPS, use metals to complete the redox reactions, through intracellular oxidase mechanisms and generate protons which decrease the pH of the solution. These reactions alter the sorption characteristics of metals and their solubilities; and hence HMs embedded in sludge may gradually change from organic fraction to the free ionic form in the solution (Yang et al., 2020). Example of direct influence is given in Eq. 1. In this mechanism sulfide is oxidized and soluble form of the metal is generated.

$$MeS + O_2 \rightarrow MeSO_4 \qquad (1)$$

In the indirect way, the redox reactions occur through other species rather than metals (Figure 1.3). These reactions may change the acidic conditions or may initiate the generation of other new species that can make complexation with metal presented in the medium and change its initial properties (Camargo et al., 2016). As it is shown in the following equations, indirect mechanism for metal solubilization, initiated by the oxidation of elemental sulfur (S^0) and the metal solubilization is occurred due to low pH, caused by the generation of acid.

$$H_2O + S^0 + 1.5\ O_2 \rightarrow H_2SO_4 \qquad (2)$$

Organic floc—Me + H_2SO_4 → Organic floc—$2H^+$ + $MeSO_4$ (3)

Revealing the exact mechanism governing the entire processes is under the investigation.

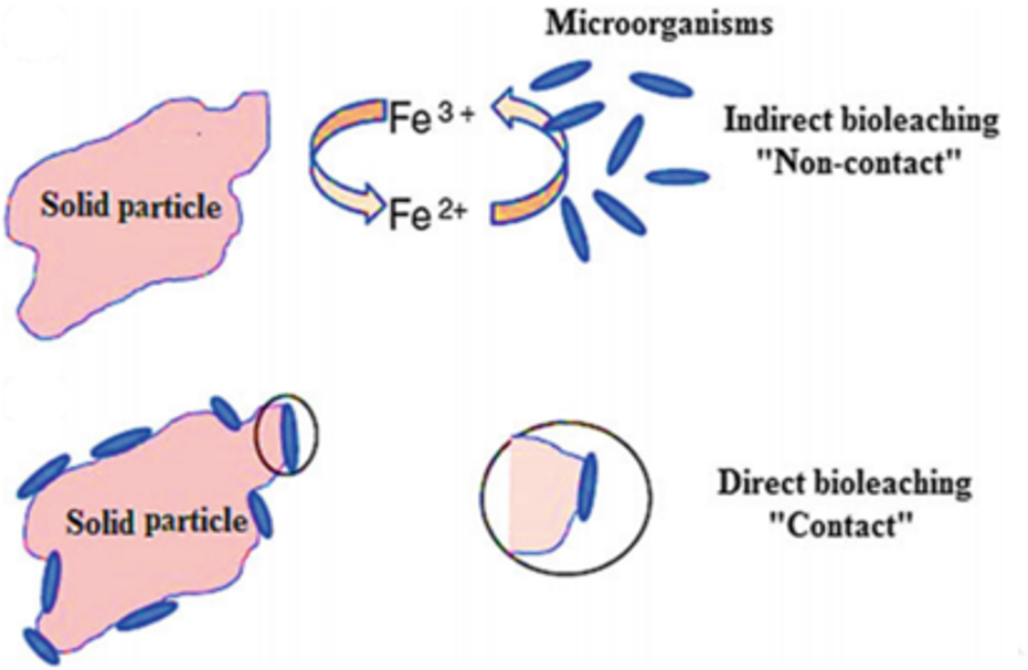

Figure 1.3. Direct and indirect mechanisms presented during the bioleaching process

(Anjum et al., 2012)

Identification of the microorganisms and their ecology may reveal necessary information required for understanding their nature and behavior. A wide range of microorganisms, e.g., chemolithoautotrophic bacteria, heterotrophic bacteria, archaea and fungi, have been identified in successful bioleaching processes (Pathak et al., 2017). Among several microorganisms *Acidithiobacillus* genus, *A. ferrodxidans,* and *A. thiooxidans* are widely used in the bioleaching processes. *Acidithiobacillus ferrooxidans* have been intensively investigated in industrial applications (Panda et al., 2015). Most of these microorganisms have been identified as autotrophs, mixotrophs or heterotrophs. Apart from above, some microorganisms fix CO_2 and grow chemolithoautotrophically and this unique characteristics, make them interested to be employed for industrial purposes (Pradhan et al., 2008). These microorganisms acidify the medium, to provide optimal conditions for solubilization of metals (Camargo et al., 2016).

1.6.2. Anaerobic Bioleaching Processes

Developing biological processes that manage the fate of HMs is a promising method to secure the environment and public health (Selling et al., 2008). In this context, role of anaerobic microorganisms in the fermentation-based metal-bioleaching processes from the solid state of the sewage sludge is becoming an issue of great importance (Meulepas et al., 2015; Molaey et al., 2021a; Selling et al., 2008). This process can be accomplished directly by the metabolism of leaching microorganisms or indirectly by the products of the metabolism (Meulepas et al., 2015). In these processes, metal content of sewage sludge might be released in acidifying stage due to ion exchange with protons since metal solubilization is related to the proton concentration. Moreover, volatile fatty acids (VFA) may act as ligand and improve the solubility of metals and thus enhance the metals leachability. These processes are accomplished through a self-sufficient mechanism, associated with several advantages over aerobic bioleaching processes, e.g., elimination of external chemical or oxygen requirement and eliminating the loss of nutrients (Molaey et al., 2021a). The mechanism of fermentation-based anaerobic bioleaching process relies on three consecutive steps, e.g., hydrolysis, acidogenesis, and complexolysis (Figure 1.4) (Yesil et al., 2021). In these interdependent biological processes, in the hydrolysis stage, sewage sludge will be hydrolyzed into dissolved organic matters (DOM) and consecutively will be converted to VFAs. Both DOM and VFAs can act as ligands and metals embedded in sludge matrix, are presumed to be leached and make complexation with either DOMs or VFAs through soluble organo-metalic complexes in the bulk solution (Molaey et al., 2021a). Based on the above description, it can be deduced that anaerobic bioleaching is considered as a promising method for decontamination of metal-laden sewage sludge as it is simple, efficient, and economically feasible. Consequently, sludge can be disposed safely for land application as an organic fertilizer or as a soil conditioner.

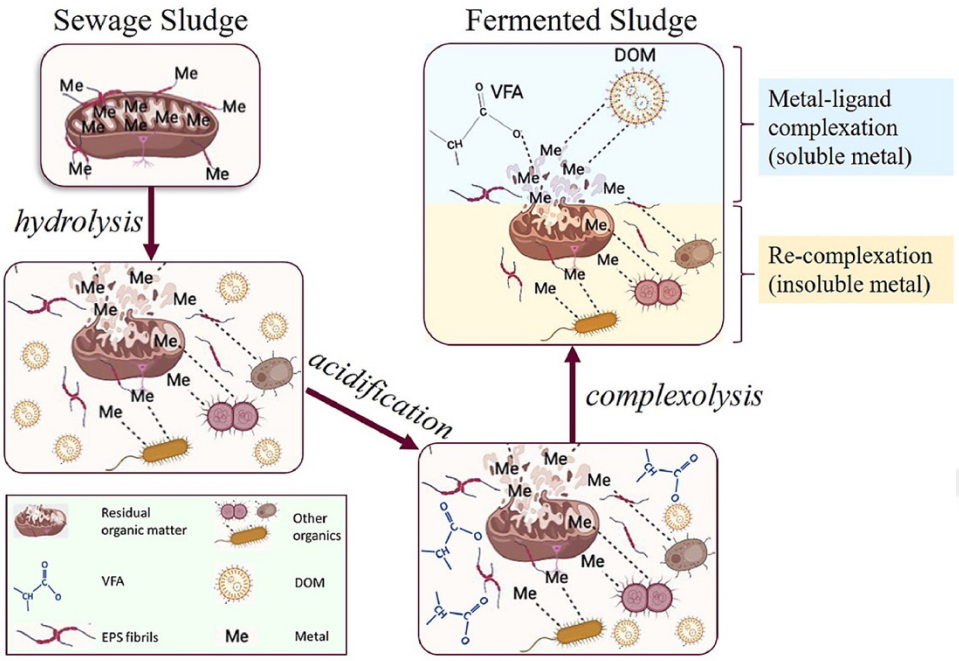

Figure 1.4. Mechanism involved in the anaerobic bioleaching system

(Yesil et al., 2021)

1.7. Parameters Affecting Bioleaching

Generally, metals on their free form are rare in sewage sludge, they exist in the complexed form of insoluble salts or embedded in the organic matter of the sludge. Metals reveal strong tendency towards organic matters embedded in sludge and therefore make strong bond to them. Moreover, owing to re-complexation of metals with organic portion of the sludge can strongly reduce the dissolution efficiency (Couillard and Mercier, 1990). Many factors can affect metal dissolution at various rates determining the overall bioleaching processes. Influences of most important parameters affecting bioleaching process are highlighted in the following sections.

1.7.1. Temperature

Microbial processes carried out within the range of physiological properties of the organisms involved in the processes. In this regard, specific growth rate of microorganisms and the superior destruction of organic matters are highly dependent on the temperature range (Tyagi et al., 1996). Therefore, temperature plays an important role in providing the optimal conditions for different types of microbial communities in the reactors. Microorganisms that can find their way toward

bioleaching of metals can be classified in three major groups based on the optimum temperature for their growth (Panda et al., 2015). Mesophilic microorganisms active and growth up to 40°C; moderate thermophilic microorganisms grow up to 55°C and extreme thermophiles growth up to 80°C (Panda et al., 2015). If temperature exceeds the maximum temperature range of microbial growth, biological processes may fail.

Thermophilic microorganisms are known by their high metabolic rate and high organic matter degradation and generally conducted at elevated temperature of 50°C. One of the common issues is the high production of total ammonia nitrogen (TAN) due to high metabolic activity and hence high degradation of organic matters. TAN is generally existing simultaneously as ammonium ion (NH_4^+) and in the free form (FAN). The later one considered as the main inhibitor due to diffusion through cell membranes and disrupting cellular activity (Molaey et al., 2018). In thermophilic microorganisms high sensitivity of cell walls and lower tolerance to high FAN concentrations are the drawbacks of using these microorganisms in the biological processes (Panda et al., 2015; Pradhan et al., 2008). On the other hand, in AD processes, acidification of the medium and hence rapid pH drop is more evident under thermophilic conditions. For indirect HMs bio-leaching decrease of pH due to metabolism is critical and due to sensitivity of thermophilic microorganisms, pH may not decrease further, once the pH declined to 4. Moreover, microbial growth might be affected when metal concentration exceed inhibitory levels (Clark et al., 2006).

Adaptation to the inhibitory level of metals is the unique properties, that have been found in mesophilic microorganisms (Chen and Pan, 2010; Panda et al., 2015). Moreover, the abundances and diversity in the microbial community have been found to be in the temperature range of mesophiles (Bárcenas-Moreno et al., 2009). Additionally, the operation of biological reactors at mesophilic condition is more stable and less energy is required compared to thermophilic temperatures. From the above description, it can be deduced that the growth rate of the leaching microorganisms plays an important role in the bioleaching processes. Therefore, HMs bioleaching processes, strongly correlated to the temperature of the bioreaction.

1.7.2. Metal Interaction with Microorganisms

Microorganisms have the ability to degrade organic matters under various conditions, and hence revealing the interaction mechanism of metals with microorganisms is of great importance.

Microorganisms can enzymatically interact with metals via metabolic processes. Manipulating this interaction, may help the remediation of environment, waste stream and some certain organics that have been contaminated by metals. Complex surface structure of microorganisms enable adhesion abilities (Wu et al., 2012), which may provide the basis for development of a new approach for HM leaching process. HMs might be presented in the EPS, cell walls, membranes, or in the intracellular fraction of the microbial community. Metals primarily bound to carboxyl-, phosphate-, and hydrosulfide-like ligands, available in the extracellular polymers, cell walls and membranes, and intracellular fraction, respectively. Moreover, microorganisms have anionic charge such as carboxylate ($-COO^-$); phosphate ($-HPO_4^-$); amino ($-NH_3^+$); sulfhydryl (-SH) and phenolic groups ($-C_6H_4OH$). They may also have hydrogen bonding properties due to presence of hydroxyl groups (-OH) (Harrison et al., 2007). Bacterial cell membranes are the main parts show the ability of cell wall to bind HMs (Figure 1.5). Metals have also show affinities to bind with oxygen containing ligands and/or with nitrogen or sulfur containing ligands (Papirio et al., 2017) . Presence of metals in microbial cell surface is correlated to the interaction of metals with phosphoryl groups and carboxylic functional groups (Mullen et al., 1989). Presence of amine groups also contribute to the fate of metals during the digestion process (Kang et al., 2007). Interaction of metals with microbial community also depends on survival of the microbes as binding mechanism with dead and alive cells are different. The sorption process of HMs with living cells can occur either through interaction with amine and sulfhydryl functional groups on the bacterial cell wall or intracellular accumulation through transport phenomena. Whereas, above mentioned surface functional groups are denatured in dead cells and thus such interactions will not occur (Gadd, 2009, 2008). In this regard, metal extraction from living and dead cells, results in different metal desorption efficiency. Because intracellular accumulation is the main mechanisms of living cells, extraction of metals from living microorganisms is low. In this regard, efficient extraction might be achieved by microbial cell disruption (Costley and Wallis, 2001; Wong et al., 1993).

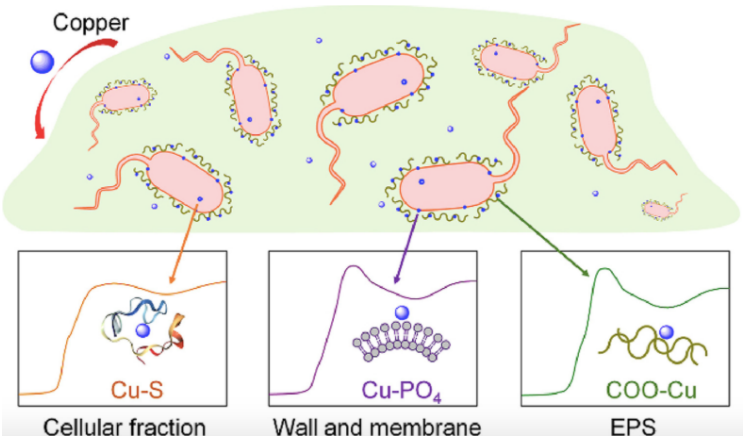

Figure 1.5. Distribution of potentially toxic metals in different parts of the microorganism (Lin et al., 2020)

1.7.3. Interaction of Metal with Extracellular Polymeric Substances

Biological activities such as; secretion, cell surface shedding and cell lysis generate extracellular polymeric substances (EPS). High proportion of organic matters and small amount of humic and nucleic acids are involved in EPS (Sponza, 2002). The abundance of the negatively charged functional groups in EPS, make them favorable places for adsorption of metals (Ledin, 2000; Wang et al., 2014). Generally, EPS divided into two main groups: bound and soluble. The former one is generated by microbes and strongly attached to the microbial community (Figure 1.6) (Papirio et al., 2017). The later one is the combination of hydrolyzed matters from organic compounds, released organic materials by cell lysis and generated soluble protein by microorganisms (Comte et al., 2006). Besides different properties, they have different binding efficiency. Complexation processes of EPS:metals can be explained by inner and outer-sphere complexes that are generated through binding metals with EPS (Avery and Tobin, 1993). In this way, interaction of metals with EPS take places through number of processes, e.g., ion exchange, surface precipitation and complexation (Figure 1.7) (Li and Yu, 2014). Interaction of metals with EPS through ion exchange process, occurs at alkaline conditions due to low concentration of protons competing with metals for available binding sites of EPS (López et al., 2000). In this context, due to involving better ion exchange properties, soluble EPS provides better conditions for the entrapment of the metals. However, tendency of metals to bind to the functional groups of EPS depends on pH of the medium (Salehizadeh and Shojaosadati, 2003), at alkaline pH due to

metal precipitation as hydroxide, the interaction of metals with EPS may decrease (Papirio et al., 2017).

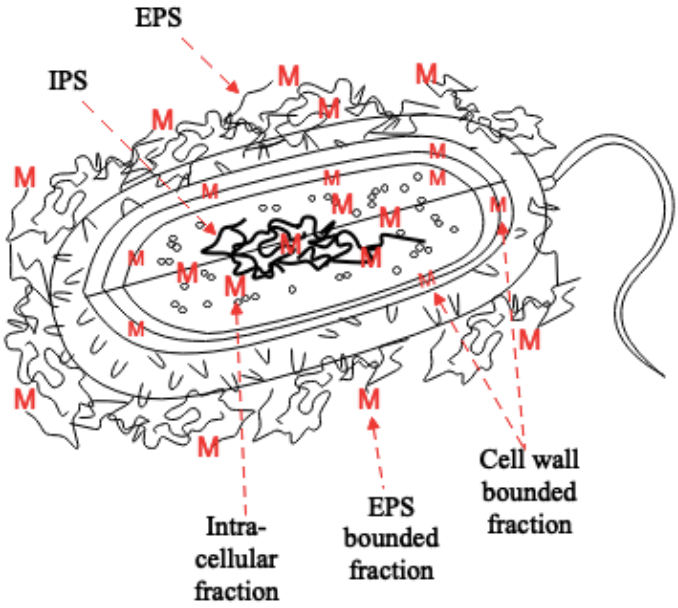

Figure 1.6. Interaction of EPS with potentially toxic metals in sludge particulate (EPS: Extracellular polymeric substances; IPS: Intracellular polymeric substances; M: Potentially toxic metal)

With presence of polysaccharides, proteins, nucleic acids in the microbial cells and EPS, abundant active groups for metal binding could be provided. For instance, polysaccharides involve number of carboxyl groups, metallothionein containing sulfhydryl groups, and phospholipids containing phosphoric acids groups. These functional groups are potent places for adsorption of HMs, thus influence the migration and transformation of HMs (Lin et al., 2020). Moreover, concentrations of metals and presence of other metals at the same time also affect the interaction of metals with EPS (Papirio et al., 2017).

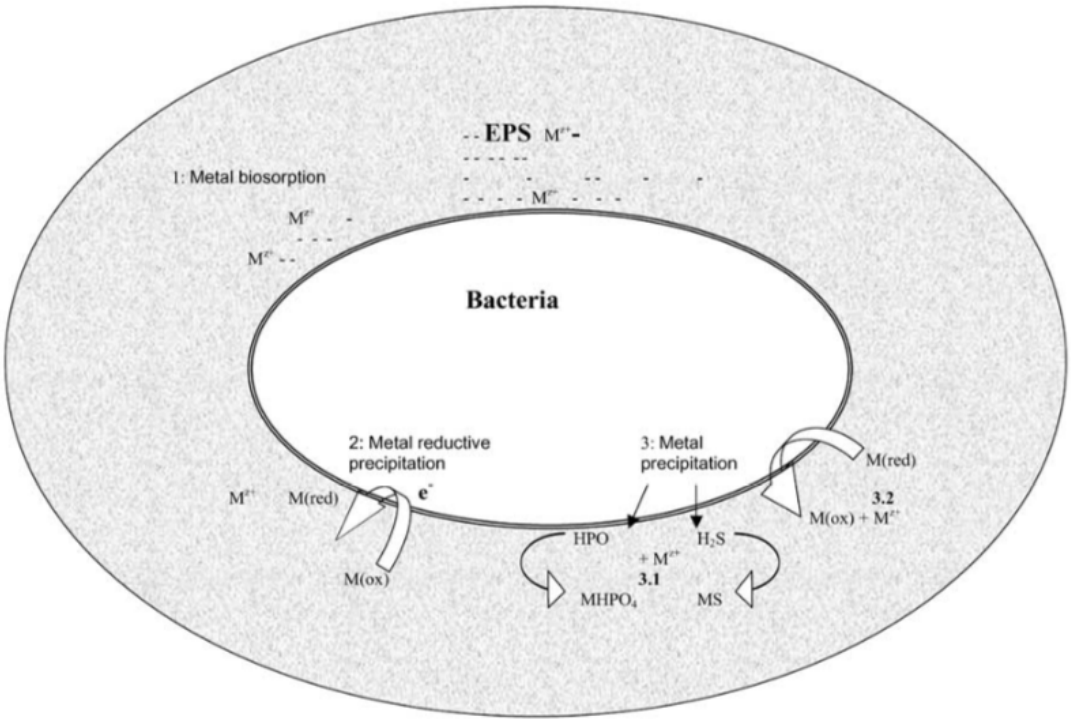

Figure 1.7. Representation of the interaction of HM with microorganisms. 1: HMs biosorption to EPS, 2: Reductive HM precipitation by enzymatic transformation, 3: 3-1 HM:sulfide and/or HM:phosphate precipitation, 3-2 Biogenic precipitation

(van Hullebusch et al., 2003)

1.7.4. Influence of Dissolved Organic Matter in the Anaerobic Bioleaching of Potentially Toxic Metals

DOM is defined as compounds involving variety of water-soluble organic matters generated during the digestion processes. DOM is mainly consisting dissolved organic carbon (DOC) and the other major elements are N, H, O, P and S (Borggaard et al., 2019). It can be fractionated into high- and low molecular weight protein-like substances, high- and low molecular weight saccharide-like substances, and humic acid like substances (D. Li et al., 2016). In AD process, hydrolysis and acidification of sewage sludge are the required steps prior to generation of value-added biomaterials or energy recovery. Within these steps, complexed organic matter are hydrolyzed to DOM that is common substrate for fermentative bacteria generating VFAs (Appels et al., 2008). In the literature it has been proved that characteristics and transformation of DOM play an important role in the VFAs and biogas production (Dai et al., 2017; Ma et al., 2019).

Moreover, with the microbial degradation and dissolution of organic matters, level of DOM in the solution will increase. Because bioleaching process, relies on dissolution of metals, and hence due to strong interaction of DOM with HMs, leachability of metals in the solution will increase (Figure 1.8). To increase the dissolution of HMs, and hence to elevate the bioleaching efficiency and rate, DOM have to be increased in the biological environment during the digestion processes. Complexation is playing a main role for the leachability of metals by DOM. Presence of complexing group, i.e. mainly carboxylate is considered to ascertain the potential of DOM to enhance the dissolution of HMs (Borggaard et al., 2019; Strobel, 2001; Weng et al., 2002). Metal:DOM associations can be occurred through mono- and bidentate complexes (Figure 1.8).

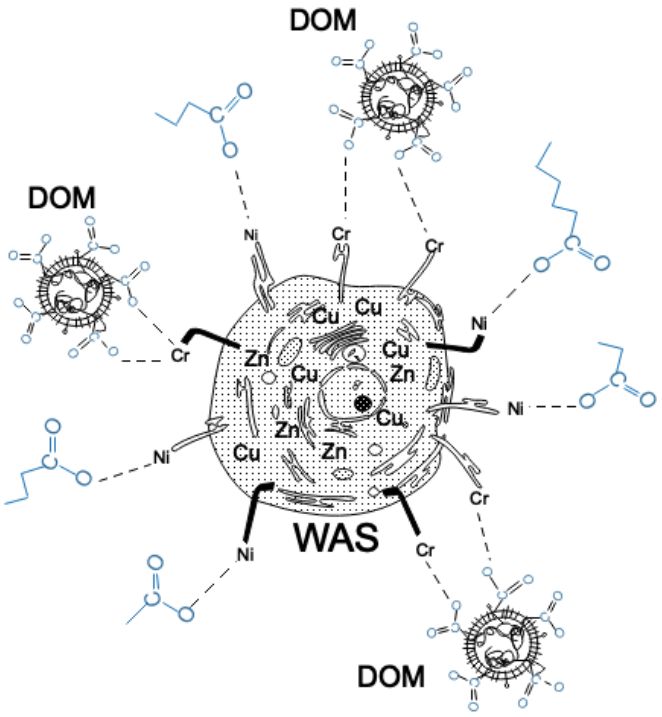

Figure 1.8. Influence of volatile fatty acids and dissolved organic matter in the solubilization of potentially toxic metals in the anaerobic digestion processes

Complexation through monodentate between functional groups within DOM and metals can be described by following equation:

$$R1R2R3CCOO^{-} + Metal^{2+} \leftrightarrow R1R2R3CCOO{:}Metal^{+} \qquad (4)$$

Complexation of bidentate between divalent metal cations and a divalent aromatic or carboxylate anions or divalent aromatic carboxyphenolate anions might be illustrated by the following equations:

$$R1R2C(COO)_2^{2-} + Metal^{2+} \leftrightarrow R1R2C(COO)_2Metal \quad (5)$$

$$R1R2R3R4C_4(COCCOO)^{2-} + Metal^{2+} \leftrightarrow R1R2R3R4C_4(COCCOO)HM \quad (6)$$

In these equations, R1…R4 representing H and/or various types of aliphatic and/or aromatic groups.

Strength of the complex is a function of the metal and the conditions of the surrounded environment (Antoniadis et al., 2017; Karna et al., 2017; Soleimani et al., 2010). Generation of the chelates, where metal cations are complexed to carboxylate groups, enhances the dissolution of metals. The ability of DOM in relation to extraction of metals seem to be related on DOM concentration, the complexing groups within DOM, metal concentration, and existence of other cations that can make complexation with DOM. Presence of several other cations; other than metals, will decrease the complexation efficiency of metals with DOM. Because any cations can bind to the functional groups of DOM and will decrease the number of these functional groups to be used for complexation of target metals (Papirio et al., 2017). Moreover, presence of anions; other than functional groups of DOM, may increase their complexation with metals and adversely affect the complexation of DOM with metals.

1.7.5. Influence of Volatile Fatty Acids and pH on the Leachability of Potentially Toxic Metals

The acid dissociation constant, measure the tendency of an acid to release proton (H^+) in the aqueous phase and produce a hydronium ion (H_3O^+). Strength of the acidity can be described by the following reaction:

$$HA + H_2O \leftrightarrow H_3O^+ + A^- \quad (7)$$

Strong acid will spontaneously dissociate in solution forming hydrogen ion and the salt. Whereas, weak acids very slowly dissociate in solution. The equilibrium constant defines the dissociation of an acid that can be described as a ratio of concentration of the species presented in the solution.

$$Ka = [H_3O^+][A^-] / HA \qquad (8)$$

The Ka values varies by orders of magnitude; and hence it necessitates to refer such data on a logarithmic value that can be described by following equations:

$$pKa = - \log_{10} Ka \qquad (9)$$

pH and pKa have strong relationship as the pH measures the concentration of protons (H_3O^+) in the solution.

$$pH = - \log [H_3O^+] \qquad (10)$$

VFAs in the biological systems present in both dissociated and undissociated forms and the degree of ionization is controlled by pH and acid dissociation constant of each acid. When pH of the environment is lower than the VFAs pKa value, more VFAs will be dominantly in non-dissociated form (Figure 1.9) (Constable et al., 2017). Whereas, when the pH is above the pKa, VFAs will be dominantly in their ionized, dissociated forms. Anaerobic bioleaching of HMs can be accomplished via ion exchange in which hydrogen ion plays an important role. Moreover, anaerobic bioleaching processes can be achieved via anionic reactions, which are dominated by the ionic forms of the VFAs (Meulepas et al., 2015; Molaey et al., 2021a). Based on the above explanation, HM leaching is correlated to coordinative ability of VFAs and metal ions. It has been proved that coordination of organic acids and HMs is related to the quantity of carboxyl functional groups (Ma et al., 2008, Gao et al., 2002; Qin et al. 2004, Shan et al., 2002 from Yan et al). Therefore, in the anaerobic digestion processes, elevated concentrations of carboxyl groups is beneficial in terms of leaching of HMs, leached metals form a complex with carboxylic acids and a stable ligand is formed.

Ligand effect of VFAs might be determined from their proton affinities. They have high intrinsic proton affinity. The oxygen atoms in the anions of carboxylate functional groups of these acids,

have low ionization energies (Panina et al., 2002). In this context, carboxylate functional groups of the VFAs are capable of monodentate coordination with divalent metal cations (Eq. 4) (Panina et al., 2002). Thus, extraction of metals from WAS using VFAs through formation of metal:carboxylate, may increase the release of HMs from WAS through competition for existed adsorption sites of organic flocs presented in the WAS (Eq. 11).

$$R–COO^- + Metal^{2+} \leftrightarrow R–COO:Metal^+ \quad (11)$$

Accordingly, interaction between organic acids and metal cations results in the formation of soluble metal:organic acids and prevents the precipitation of metals. This phenomenon can be explained by ion exchange with protons; thus, the dissolution of cationic metalloids is related with the proton concentration. Functional groups of the organic acids, gradually dissociate starting from pH 4 and the dissociation processes completed at pH 7. The availability of the carboxylate groups ($-COO^-$) as a result of dissociation of organic acids can react with metals and keep them in the solution (Zhu et al., 2014). It can be deduced that formation of chelates, where metal cations are bonded to carboxylate group enhances the solubilization of metals. Therefore, metal:organic acid interaction seems to be dependent on the concentration of organic acids and contents of carboxylate functional groups as well as concentration of metals and other cations that can interfere the complexation of metals:organic acids. To obtain good interaction of metals with organic acids and to enhance anaerobic bioleaching efficiency, significant amount of VFAs but only small amounts of metals should be presented in the system. pH significantly affects the metal biosorption processes and is generally considered as one of the most important factors for managing bioleaching processes. Optimum pH can enhance the dissolution efficiency as the equilibrium shifts in the favor of dissolved metal formation. Generally, the highest solubility of metals in the solution can be achieved at low pH, represses microbial activities and enhances the competition among metallic cations for binding to the adsorption sites; and hence decreasing the metal biosorption efficiency. As the pH increases, the bioleaching efficiency of metallic cations decreases and may enhance the metal precipitation at pH above 7 (Papirio et al., 2017). Due to cooperation of various type of microbial species in the bioleaching of HMs, the process can be initiated at neutral pH (Blais et al., 1992). In AD process, hydrolytic microorganisms, are favored to hydrolyze organic matter at neutral pH. In the subsequent degradation processes, acidic environment will be provided that might be the favor of another types of microorganisms presented

in the digestion processes. Therefore, with initiation of bioleaching process at neutral pH, without pre-acidification of the medium, high chemical consumption will be prevented, and hence the operational cost of the process will be reduced.

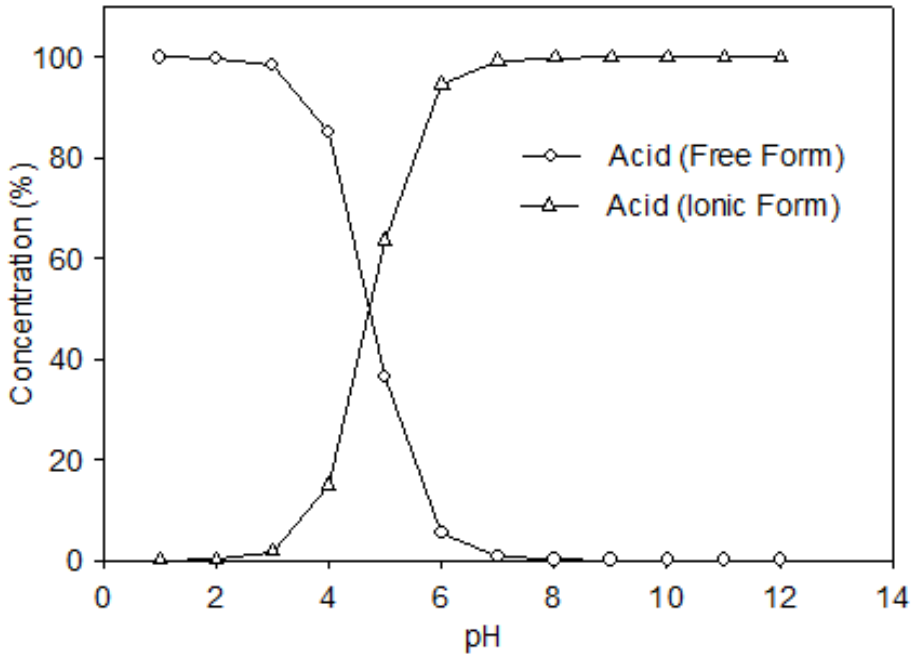

Figure 1.9. Ionization of volatile fatty acid with respect to variation in pH values

1.8. Post-treatment of the Effluent Digestate

Sewage sludge is a heterogeneous system involving microbial communities that are the parts of the biopolymeric network (Wu et al., 2017). As described previously, metals are easily adsorbed in the sludge structure systems via complexation and neutralization via negatively charged functional groups in the bio-polymeric systems. Some chemical and biological methods have been proposed to remove metal content of the sewage sludge, e.g., chemical chelating agents (Pei et al., 2016), application of fungal microorganisms (Rezza et al., 2001) and using chelated-Fe^{2+} catalyzed Fenton process (Pathak et al., 2009).

EPS comprising protein, polysaccharide, lipids and small amount of uronic and nucleic acids, etc. They are considered as the main organic matters in the sewage sludge containing 60 to 80% of

sludge organic matter and have significant influence on the sludge structure, settling, and surface charge characteristics (Tang et al., 2020). The abundancy of negatively charged functional groups within the EPS, make them potent sites for adsorption of metals. The binding processes with metals are accomplished via electrostatic attraction, ion exchange, surface precipitation, complexation, etc. Therefore, EPS have crucial effect in transformation of HMs (Xu et al., 2019). Anaerobic digestion of sewage sludge is generally utilized for its ability to stabilize sludge and energy production in the form of methane. Therefore, in order to increase methane production potential through anaerobic digestion, researchers concentrate on developing extensive pre-treatment process such as thermal, mechanical, chemical and biological processes (Y. Li et al., 2016; Neyens and Baeyens, 2003; Wang et al., 2013; Xu et al., 2019). However, as far as metal removal is concerned, introduction of pre-treated effluents from these processes to AD process may generally decrease the mobility of metals. As described formerly (Section 1.7.3), the lower solubilization of metals can be described by strong binding tendency with sewage sludge as a results of metal's extracellular precipitation, cell surface sorption and intracellular accumulation (Papirio et al., 2017). Metals bounded to organic substances have strong influence on the stability of the structure of sludge particulate (Xu et al., 2018). Moreover, stable structure of the sewage sludge is resulted from interaction of EPS with microbial cells, other EPS molecules and other particles embedded in sludge (Sponza, 2002; Xu et al., 2019). Therefore, sewage sludge have been directed to extensive pre-treatment processes, to enhance the biogas production rate and efficiency, (Y. Li et al., 2016; Neyens and Baeyens, 2003; Wang et al., 2013; Xu et al., 2019). Eventually, organic flocs underwent a degree of decomposition; meanwhile, HMs may migrate and transform (Xu et al., 2019). Introducing subsequent effluents from these processes to AD processes may decrease metal solubilization due to biosorption mechanisms with both metabolic and non-metabolic microbial activities (Papirio et al., 2017). Biosorption of metals can be described with metal binding capacity to the cell biomass as a results of metals extracellular precipitation, cell surface sorption and intracellular accumulation (Papirio et al., 2017). Based on this argument, it can be deduced that solubilization of metals can be facilitated by post-treatment of anaerobic digested sewage sludge due to low levels of organic matters in the digestate compared to undigested sludge. Although, post-treatment is required for safe disposal of digestate in arable land, surprisingly little attention has been paid to post-treatment of effluent digestate for removing HMs from the solid state of the digestate.

To degrade organic portion as well as EPS, advanced oxidation process as a post-treatment is predicted to directly attack the EPS. Decrease adsorption capacity of the sludge as a result of EPS degradation, can be followed with increase in metals release efficiency. During the oxidation process metals are expected to migrate into the liquid fraction and thus remove them through solid-liquid separation. Fenton's reagent (H_2O_2 / Fe^{+2}) has been proposed as an alternative oxidation process for disruption of organic flocs in the digestate. For instance, hydroxyl radical produced through Fenton reaction can break-down and disrupt the EPS. Therefore, extracellular and intracellular biopolymers which retain large number of metals, undergo high degree of decomposition under the oxidation process. Meanwhile, HMs may migrate and transform into the soluble fraction during this process. Sonication is considered as one of the another efficient methods for disintegration of sewage sludge by strong shear forces that attack the flocs and facilitate the destruction of the flocs (Zhao et al., 2016). Simultaneous application of ultrasonic with aeration can significantly decline the energy consumption. Aeration is a cost-effective and benign alternative and thus air-assisting ultrasonication is a promising method to improve the sludge disintegration (Molaey et al., 2021b).

1.9. Aim of the Thesis Study

Land application of stabilized sewage sludge has been considered as a cost-effective method for its disposal. However, mainly depending on the composition of the wastewater, sewage typically contains a diverse range of recalcitrant and potential hazardous contaminants such as adsorbable halogenated organic compounds (e.g., PFAS, AOX), phenols, sulphates, aromatics, API's (e.g., antibiotics, endocrine disruptors), pathogens (viruses, bacteria, etc.), microplastics, and HM. Although the land application of stabilized sewage sludge can be an environmental concern due to all the listed micro-pollutants and heavy metals, only the heavy metal removal from sewage sludge was considered in this study. Heavy metals are non-biodegradable; therefore, metal content of the sewage sludge restricts its direct application into the soil as an organic fertilizer. In AD process, particulate organic matter will be hydrolyzed and as a consequence of fermentation processes, VFAs will be generated. Accumulation of VFAs in the system, induces the drop of pH and it is predicted to accelerate the solubilization of metals and the solubility may further increase by complexation of metals with VFAs. Therefore, bioleaching efficiency is dependent on the biological conversion of organic matter to which metals are adsorbed or complexed in. On the

other hand, sewage sludge comprises substantial content of organic matters, e.g., proteins, carbohydrates and lipids. Owing to complex and rigid structure of sludge flocs, the solubilization process are slow that limits the release of organic matter for subsequent acidification. Sludge organic substances are mostly composed of extracellular organic substances and cell biomass that reinforced the sludge against biological eradication. Moreover, HMs tend to make complexation with organic content of digestate, that makes a great problem for disposal or further use. Besides that, metals bound to organics, which account for substantial proportion of metals in sludge, limits bioconversion of WAS, and when their concentrations increase, WAS biological degradation decreases. This can be described by the EPS content of WAS that have different adsorption sites, such as organic moieties, aliphatic groups and hydrophobic regions. Metal ions mainly existed as cations, which increased the complexation ability to organic matters. Based on the divalent cation binding theory, functional groups such as carboxyl, hydroxyl, etc. embedded in EPS, can form a bridge between organic compounds and metals and increase their recalcitrant properties against biological degradation (Xu et al., 2019). Hence, to continue the use of agricultural land disposal of digestate, reducing toxic metal content of the digestate is mandatory. It is predicted that AD may increase bioleaching of metals to some extent. However, the significant portion of the toxic metals would still remain in the digestate after the digestion. Cells are the major part of organic matter in digestate flocs and EPS have been reported to be outside of cells and attached tightly to the cell membrane. Due to existence of negatively charged functional groups, higher biosorption of metals can occur mainly due to interaction of metals to EPS. Release of EPS and intracellular organic matter could occur through cell lysis under oxidizing agents. Hence, release of metals from sludge to the bulk liquid can be improved via destruction of sludge flocs. However, because limited bioleaching occurs during conventional AD process, anaerobic digestion of sewage sludge integrated with post-treatment is required for safe disposal of digestate in arable land and surprisingly little attention has been paid to study the combination of biological, chemical and/or physical processes for HM removal from the solid state of the digestate. Therefore, the objective of this study was to investigate the continuous anaerobic digestion of sewage sludge with co-substrates at various OLRs to reveal the migration and transformation behavior of HMs. Readily biodegradable organic carbon such as sugar and carbon rich substrates such as grass were used as co-substrates. In this study, Fenton's reagent (Fe^{2+}/H_2O_2) as an alternative chemical post-treatment was applied to increase the solubilization of HMs. Generated hydroxyl radicals through Fenton's

reaction were predicted to degrade EPS and thus increase the solubilization of HMs. Moreover, in this study, integration of sonication with aeration as a post-treatment is predicted to efficiently decompose the EPS and increase the solubilization of metals. Although, sonication is very efficient method for sludge decomposition, due to high energy consumption of ultrasonic sludge disintegration, air-assisted ultrasonication can be used to enhance the decomposition efficiency and decrease the energy consumption (Zhao et al., 2016). During air-assisted ultrasonication, more cavitation bubbles will be generated with strengthened cavitation effect, causing break down of EPS and thus resulting in the solubilization of metals. To the best our knowledge, this is the first study investigating the Fenton's reaction and air-assisted ultrasonication as a post treatment to remove HMs from the solid fraction of anaerobically digested sewage sludge (Figure 1.10).

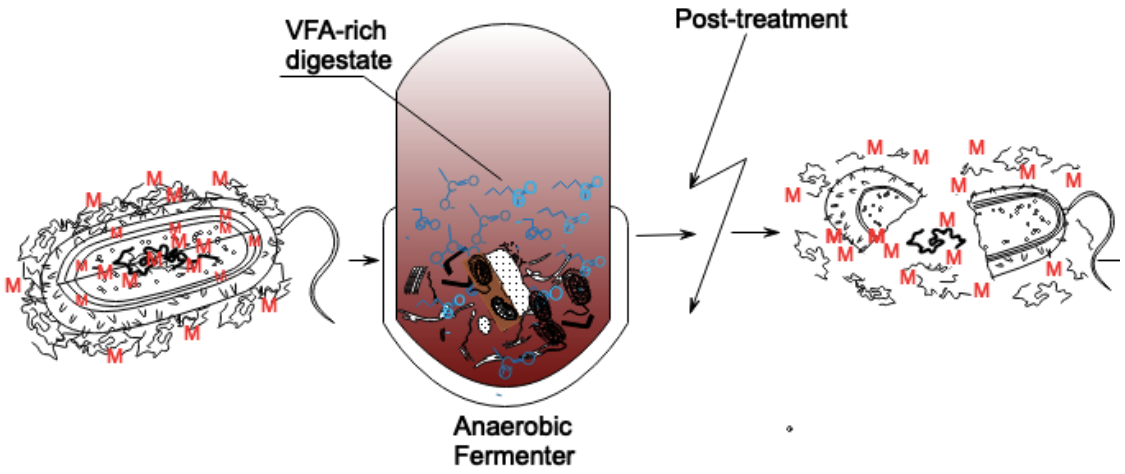

Figure 1.10. Proposed mechanisms for efficient dissolution of potentially toxic metals from sewage sludge

2. MATERIALS AND METHODS

2.1. Substrate and Inoculum

The inoculum used in the experiment were taken from a 5-L laboratory-scale mesophilic anaerobic fermenter fed with dog food. Dried and thickened waste activated sludge was provided from Paşaköy and Ataköy advanced wastewater treatment plants, located in Istanbul, Turkey. Thickened waste activated sludge was stored in 20 L barrels at 4 ^{o}C prior to each feeding. As a co-substrate, grass was collected in summer from recreational areas (Göztepe Bridge) located in Kadıköy, Istanbul, Turkey. Characteristics of the substrates and inoculum are given in Table 2.1.

2.2. Reactors Set-up and Operation

In the first part of the study, two identical completely mixed lab-scale biogas reactors (R1 and R2) with total volume of 6 L and active volume of 5 L operated at 36±1 ^{o}C (Figure 2.1). Based on OLRs, the reactors were operated at 5 different phases (Figure 2.2). In phase I, the SRT was 8 days and in other phases the SRT was kept constant to 4 days. Dried WAS from Paşaköy WWTP was used as a substrate in both reactors.

Table 2. 1. Physico-chemical properties of the WAS and inoculum

Parameter	Paşaköy WAS	Ataköy WAS	Grass	Inoculum
Total solid (TS) (g/kg sludge)	963.28±4.23	104.38±0.49	264.33±1.52	27.07±6.02
Volatile solid (VS) (g/kg TS)	592.18±12.81	59.27±0.91	237.01±1.46	24.66±6.73
Total Kjeldahl nitrogen (TKN) (g/kg TS)	52.26±0.98	36.05±2.98	10.07±0.46	28.05±0.57
Cr (mg/kg TS)	445±39	713±24	-	-
Cu (mg/kg TS)	261±10	707±3	-	-
Ni (mg/kg TS)	184 ± 16	369 ± 3	-	-
Zn (mg/kg TS)	909 ± 87	1559 ± 6	-	-

To reveal the influence of VFAs in the bioleaching of HMs, the OLRs were increased gradually from 3.81 gVS/L·d to the maximum value of 17.65 gVS/L·d. From the beginning of the study up to the end of phase III, R1 and R2 were parallel, and the OLRs in phases I, II and III were 3.81, 8.91 and 16.7 gVS/L·d in both reactors. To evaluate the effect of thermal pre-treatment, thermally pre-treated WAS was used for 40 days between days 72 and 112. However, thermal pretreatment of WAS, was terminated at day 112 because it had no great effect on hydrolysis and VFAs production. In phase IV and in R1, OLR was remained unchanged, while OLR in the case of WAS, decreased to 8.91 gVS/L·d in R2.

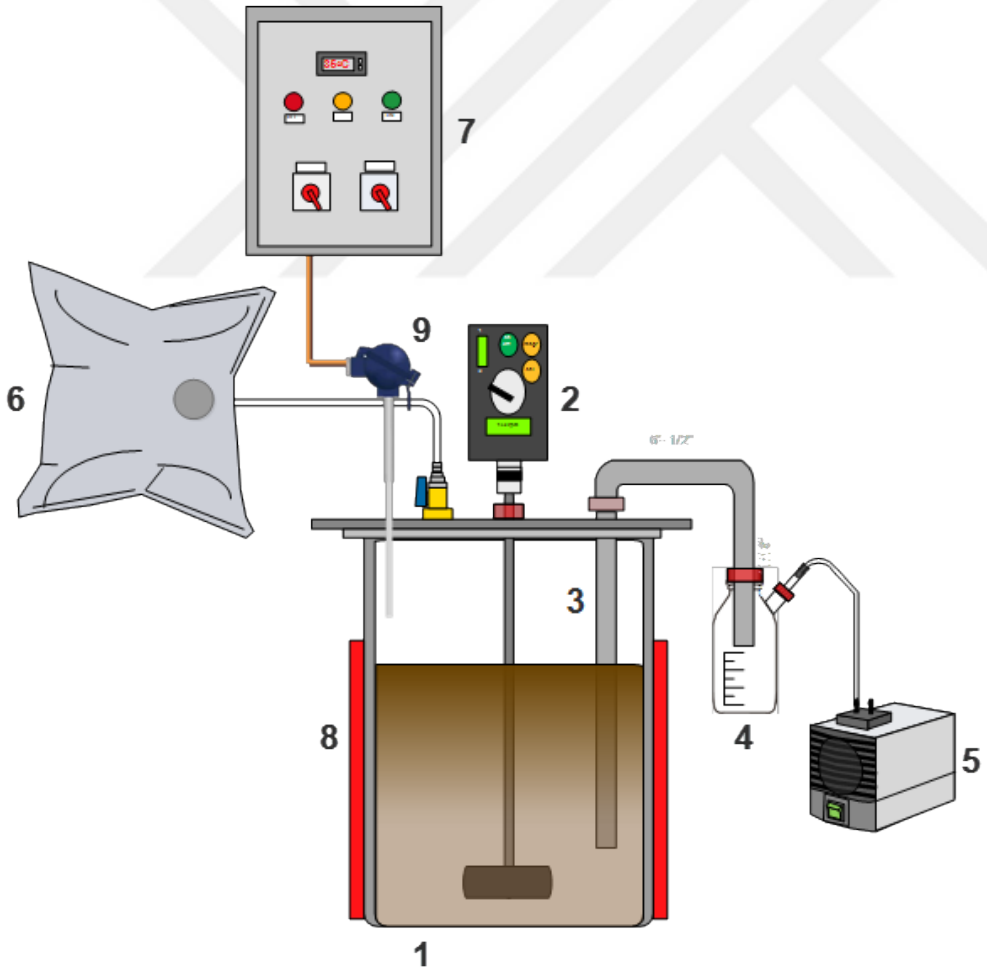

Figure 2. 1. Experimental set-up; (1) Anaerobic digester, (2) mixer, (3) feeding and effluent port, (4) effluent container, (5) vacuum pump, (6) gas holder bag, (7) temperature control unit, (8) heating coil

As a readily biodegradable COD, extra carbon source; i.e., sucrose was supplemented in R2 to equalize the COD load in both reactors and to reveal the influence of VFAs in the anaerobic bioleaching of HMs (Figure 2.2). In the last phase the OLR was increased to 17.65 gVS/L·d in both reactors while the addition of readily biodegradable organic source, e.g., sucrose into R2 was continued.

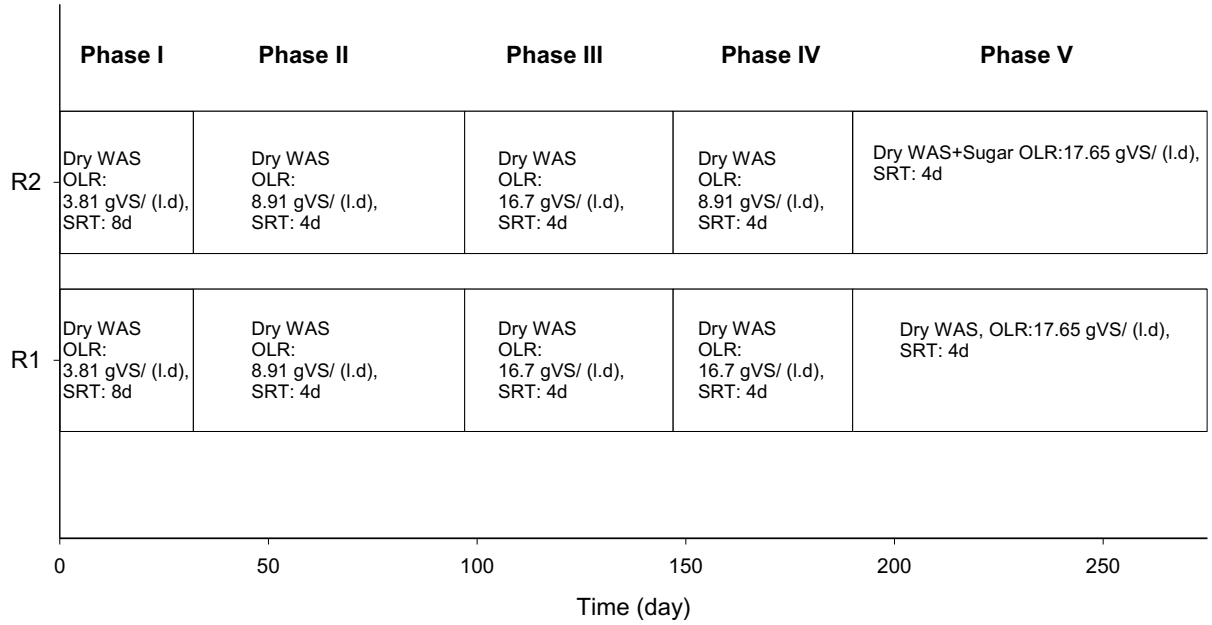

Figure 2. 2. Feeding regimes of reactors in anaerobic fermentation process (Dry WAS: Representing sludge which is taken from the drying unit of the Paşaköy WWTP; Thick WAS: Representing the WAS that is taken from thickener unit of Ataköy WWTP; US: Representing Ultrasound)

In the second part of the study, operation of the acidogenic reactors (R1 and R2) that had been operated in the first part of the study, were converted to methanogenic reactors and their operation continued for an additional 6 months and SRT was increased to 16 days (Figure 2.3). Moreover, to reveal the influence of co-substrate in the bioleaching of HMs, another reactor (R3) with the same operational conditions (OLR of 2.68 gVS/L·d and SRT of 16 days) was operated and thickened WAS from Ataköy WWTP was used as a substrate and ultrasound pretreated grass was used as a co-substrate (Figure 2.3). Experimental conditions for pre-treatment including time, power, and power density were 10 min, 150 W and 1 W/mL, respectively. Feeding regimes of R1 and R2 were changed on day 48 (Figure 2.3). In R1, thickened WAS from Ataköy WWTP was used as a substrate rather than dried WAS from Paşaköy WWTP. In R2, sucrose was replaced with

ultrasound pretreated grass as a co-substrate. From day 85, OLR of all reactors was increased to 3.02 gVS/L·d and SRT was remained like in previous phase (Figure 2.3).

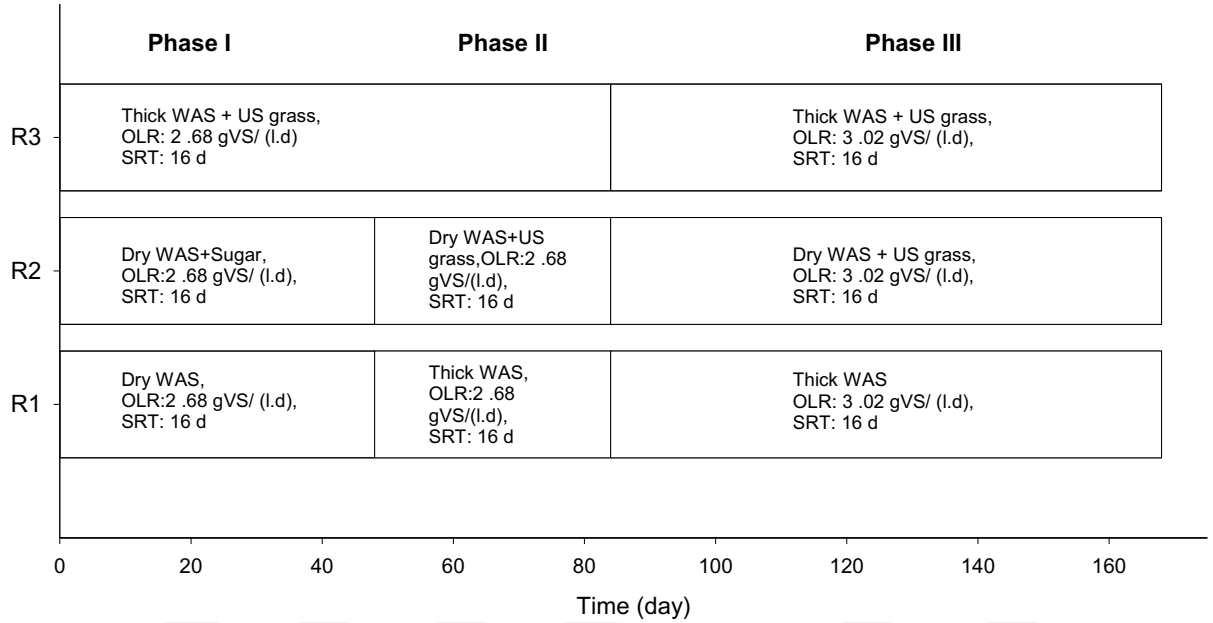

Figure 2. 3. Feeding regimes of reactors in anaerobic digestion process (Dry WAS: Representing sludge which is taken from the drying unit of the Paşaköy WWTP; Thick WAS: Representing sludge that is taken from the thickener of Ataköy WWTP; US: Representing Ultrasound)

Table 2. 2. Introduced substrates into the methanogenic reactors at different phases

Reactors	Phases		
R1 R2 R3	Phase I	Phase II	Phase III
Substrate	D.WAS [(1)] D. WAS+US grass Th.WAS+US grass[(1)]	Th. WAS D.WAS+US grass Th. WAS+US grass	Th. WAS D.WAS+US grass Th. WAS+US grass

[(1)] D. WAS: Representing dried waste activated sludge; Th. WAS: Representing thickened waste activated sludge

The digestate was analyzed for VFAs, pH, TS and VS, total alkalinity, total ammonia (TAN), total and soluble metals, total and soluble chemical oxygen demand (COD) once a week.

Biodegradation efficiency of reactors was determined by the ratio of soluble COD/total COD. Daily biogas produced was collected in aluminum-foil gas bags and the amount was measured with water displacement system. Paddle mixers used to agitate the digester at 90 rpm.

2.3. Post-treatment of Digestate Using Advanced Oxidation Processes

In each experiment 50 mL of digestate was transferred into the test tubes. The pH was adjusted using HCl and NaOH. In the treatment processes, 88 mg/gVS of H_2O_2 and 110 mg/gVS of $FeSO_4.7H_2O$ was added to the prepared sample and were shaken for 24 hours. After completion of the post-treatment processes, samples were centrifuged for 5 minutes, the supernatant was introduced to vacuum filtration and the filtrate was used for heavy metals determination using atomic absorption spectrophotometer (Perkin Elmer AAS 400).

2.4. Post-treatment of Digestate Using Air-assisted Ultrasonication

Ultrasound was applied using an ultrasonication device (Bandelin, Sonopuls HD3200, Germany) and frequency of the sonication and amplitude were 20 kHz and 100%, respectively. Titanium probe (Bandelin, VS 70T, Germany) diameter was 13 mm and adjusted power to the subject samples was 100W. To meet the energy threshold, the cavitation effect of ultrasonic irradiation energy should be higher than 0.3 W/mL (Zhao et al., 2016). Therefore, in this study to enhance the cavitation effects, 100 mL of digested samples were subjected to sonication, resulting in a 1 W/mL density. Ice bath was used to control the temperature between 20 and 25 ^{o}C during sonication experiments which lasted for 10 min. Specific energy inputs of the sonication to the samples were calculated by multiplying the power and duration of the sonication over the sonicated sample volume and TS content of the samples. It has been found that ultrasonic disintegration of sewage sludge by aerating the sample in the last minutes of the sonication time was the most effective period for disintegration of organic flocs in SS (Zhao et al., 2016). Accordingly, in this study to utilize the gas bubbles effectively, and generate strong shear force, air bubbles were introduced in the last 2 minutes of the sonication time. Air was supplied with an aquarium pump (Eheim air pump 100, Germany) with a flow rate of 0.875 L of air/(L of digestate. min), and applied with a PVC tubing submerged into the sludge sample.

2.5. Analytical Methods

Thermal pre-treatment of WAS was conducted with an autoclave (HMC Europe, HMT 232X, Taiwan). Test temperature could be reached at 30 minutes and the thermal treatment of WAS was continued for 1 hour at test temperature of 120 ^{o}C. Sample volume was approximately 1L and to avoid the water evaporation it was placed in a closed bottle. TS, VS, pH, total ammonium nitrogen (TAN), TKN, COD, total and soluble HMs, e.g., Cr, Cu, Ni and Zn contents of the sludge samples and digestate were analyzed in triplicate and were determined according to the Standard Methods (APHA-AWWA-WEF, 2005). The digestate samples, were analyzed for pH, VFAs, HMs two times a week and alkalinity, TAN, TS, and VS measurements were conducted once a week. pH was measured using a pH meter (WTW 3310, Germany), alkalinity was determined with titration Method 2320 B (APHA-AWWA-WEF, 2005). TS and VS quantified using methods 2540 B and 2540 E, respectively (APHA-AWWA-WEF, 2005). TAN concentration was measured with nesslerization method and total TKN measured using method 4500 Norg B (APHA-AWWA-WEF, 2005). VFAs and CH_4 content of the biogas produced were analyzed using two separate gas chromatographs (Shimadzu GC-2014, Japan) equipped with flame ionization detector and thermal conductivity detector, respectively. In both analysis the methods described by Bayrakdar et al. (2017) were used. Total HMs content and liquid fractions in digestate and WAS was determined by microwave-assisted acid leaching technique (Florian et al., 1998). Total metal analysis was carried out by acid digesting 0.5 g of sample, with concentrated nitric acid in a digestion vessel using a microwave oven (CEM, MARS-5, US) as described by Molaey et al. (2018). Samples were centrifuged with relative centrifugal force (RCF) of 4.226 for 5 minutes and filtered through 0.22 μm filter paper and the filtrate was used to determine soluble fraction of HMs. Total and soluble metal contents were determined by atomic absorption spectrophotometer (Perkin Elmer AAS 400, US). Metal speciation was determined using the modified sequential extraction procedure of Tessier et al. (1979) as reported by Hullebusch et al. (2005). Based on this procedure, distribution of metals in four different fractions such as exchangeable, carbonate bounded, organics bounded, and residual was determined. Exchangeable part of metals are susceptible to variation in ionic composition of solution; carbonate bounded ones are likely to be affected by the changes in pH values; organic bounded ones can be released in oxidizing environment; and the residual fraction of metals are in the crystalline structure of solids (Dong et al., 2013; Huang and Yuan, 2016). In statistical analysis and graphical plots, Sigma Plot Software Version 12.0 (Systat Software Inc,

USA) was used. One-way analysis of variance (ANOVA) using post-hoc Tukey’ s test was implemented to reveal the significance of the results with the reliable level of 95% ($p = 0.05$).

2.6. Establishment of Visual MINTEQ 3.1 prediction for speciation of potentially toxic metals

The chemical equilibrium software, visual MINTEQ version 3.1 was used to model the distribution of HMs in digestate. It can predict chemical reactions based on the chemistry of solutions in equilibrium with other species such as minerals, and adsorptive surface of sorbent species in the solution. The inputs were total metal concentration, temperature and pH, initial redox potential of -300 mV, VFAs concentration, total alkalinity and total ammonia nitrogen (TAN). Influence of the bio-sorption, and complexation of metals with recalcitrant DOM were not included in the model due to the lack of input information.

3. RESULTS AND DISCUSSION

3.1. Potentially Toxic Metal Content and their Distribution in Different Fractions of the Sewage Sludge

Total metal, Cr, Cu, Ni, and Zn content of dried WAS from Paşaköy and thickened WAS from Ataköy advanced WWTPs effluents, along with the permissible limits for land application in EU and US national regulations are given in Table 3.1.

Table 3. 1. HMs content of the WAS from Paşaköy and Ataköy WWTPs along with the maximum permissible limits for land application in EU and US

Metal (mg/kg)	Paşaköy	Ataköy	EU[a]	US[a]
Cr	445 ± 39	713 ± 24	-	3000
Cu	261 ± 10	707 ± 3	1000-1750	4300
Ni	184 ± 16	369 ± 3	300-400	420
Zn	909 ± 87	1559 ± 6	2500-4000	7500

a: (Inglezakis et al., 2014)

Based on these data, total concentrations of target metals in both dried WAS from Paşaköy WWTP and thickened WAS from Ataköy WWTP, were below or within the corresponding concentrations in the EU and US legislations (Table 3.1). It has been stated that recurrent application of sludge resulted in HM accumulation in crops even when metal content of the sludge complied with the regulations (Singh and Agrawal, 2007). Speciation of the HMs (Cr, Cu, Ni and Zn) was estimated by the modified Tessier sequential extraction procedures (Hullebusch et al., 2005) in dried WAS from Paşaköy WWTP and thickened WAS from Ataköy WWTP and the values were represented as percent of total concentrations (Figure 3.1). It is evident that the main differences between these two types of WAS are the relative abundance of HM species in different fractions that might have been influenced by the incoming wastewater. Thus, heavy metal speciation might be varied among different WWTPs as a result of location, living habits, etc. (Figure 3.1).

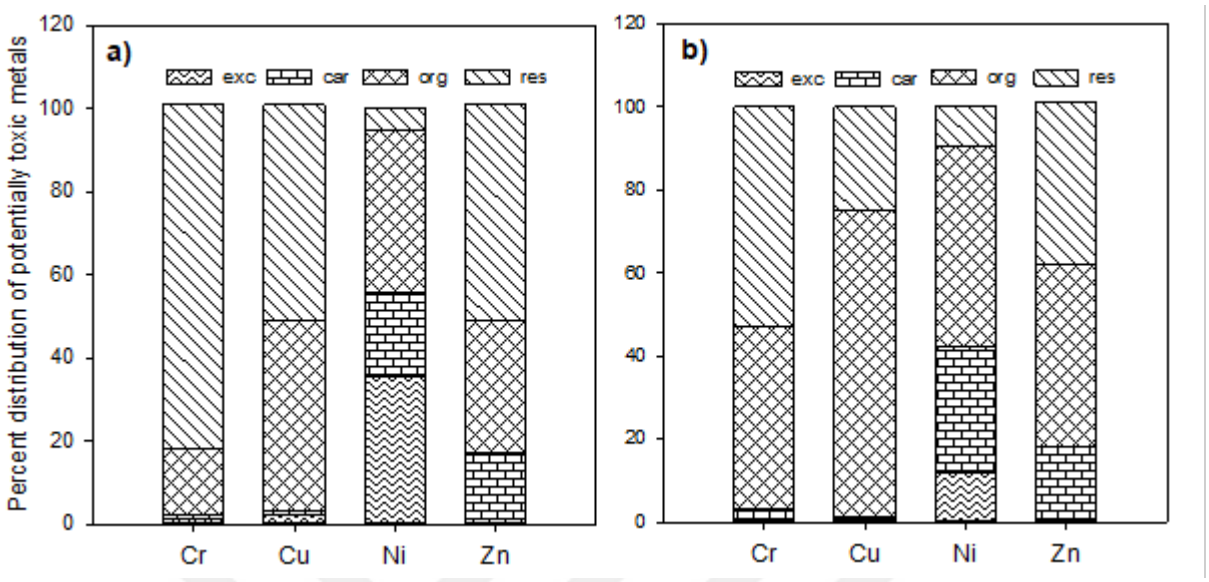

Figure 3. 1. Fractionation of metals in raw sewage sludge: Dried WAS from Paşaköy WWTP (a), Thickened WAS from Ataköy WWTP (b)

Fractionation of metals in raw WAS revealed that, except for Ni, exchangeable fractions of aforementioned metals were low in WAS, indicating less direct toxicity to the environment (Figure 3.1). The dominant binding phases for Cr, Cu and Zn were residual and organically bound fractions for both dried WAS from Paşaköy WWTP and thickened WAS from Ataköy WWTP. These results are partially consistent with the finding of Suanon et al. (2016) who highlighted that HMs are highly distributed between organic bound and carbonate fractions. In this study, sum of the organic bound and residual fractions was higher than 98% of the total concentration of Cr and Cu for both dried WAS from Paşaköy WWTP and thickened WAS from Ataköy WWTP (Figure 3.1). Sum of the same fractions exceeded 80% for Zn in both type of WAS (Figure 3.1). Distribution of Cr and Cu in residual fraction, especially in dried WAS from Paşaköy WWTP, was considerably higher than the same fraction of the raw WAS reported in the study of Zhang et al. (2016). Ni was widely distributed between exchangeable, carbonate, and organic bound fractions in both WAS samples (Figure 3.1). Sum of the carbonate and organic bound fractions of Ni, was higher in thickened WAS from Ataköy WWTP compared to dried WAS from Paşaköy WWTP. However, exchangeable fraction of Ni in dried WAS from Paşaköy WWTP was three times higher than the thickened WAS from Ataköy WWTP (Figure 3.1). High concentration of Ni existing in unstable fractions, exchangeable and carbonate fractions, were also reported in previous studies (Fuentes et al., 2008; Li et al., 2019).

3.2. Anaerobic Fermentation of WAS in Acidogenic Reactors

3.2.1. Performance of the reactors during the fermentation process

Increasing OLR gradually from 3.81g VS/L·d in phase I to 8.91 g VS/L·d in phase II, resulted in slight increase in VFAs and VFA concentration reached to 6800 mg CODeq/L on day 51. Afterwards the VFA concentration dropped to lower than 4800 mg CODeq/L on day 55. Drop in VFA concentration was attributed to the foaming formation inside the reactors, and entrapped solids in the foam developed high solid content at the top of the reactor, providing the formation of dead zone and reducing the reactor active volume. Because of the short SRT (8 days), only negligible (<1%) amount of CH_4 was detected in the fermentation gas produced. Therefore, the CH_4 percentage data were not presented in the text. Addition of thermally pre-treated WAS into the digesters starting from day 71 had minor influence in the VFA concentration and reached to 7170 mg CODeq/L on day 97 (Figure 3.2b). WAS accounts up to 40% protein, 25% lipid and 15% polysaccharide in terms of COD (Razavi et al., 2021; Wilson and Novak, 2009). In disintegration of WAS, solubilization occur due to hydrolysis of proteins and polysaccharides (Liu et al., 2012). In thermal pretreatment, macromolecule proteins are degraded to peptides and further transformed to amino acids (Gagliano et al., 2015). In addition, polysaccharides will be transformed to smaller molecules, e.g., glucose. As a result of elevated temperature, smaller molecules of carbohydrates make an interaction with amino acids and form Maillard reaction wherein carbohydrates and amino acids generating another compounds called melanoidins that are hard to be biodegraded (Carrère et al., 2010). In thermal pre-treatment, melanoidins can be generated at high temperatures (150 ^{o}C) or with longer treatment time at low temperatures (<100 ^{o}C) (Ariunbaatar et al., 2014). Accordingly, in this study, thermal pretreatment caused a dark brownish color in the WAS, indicating the possibility of Maillard reaction.

During the digestion processes, abundant VFAs in the system were in the forms of acetic acid (1590±44 mg CODeq/L) and propionic acid (1719±97 mg CODeq/L). Perhaps, acetoclastic and hydrogenotrophic methanogens were washed out from the reactor due to low SRT (8 days), leading to disruption of syntrophic acetogenesis. Consequently, both acetic and propionic acids were accumulated in the reactor. Low VFA production was also attributed to partially stabilized WAS, which was taken from an extended aeration activated sludge system operated at long SRTs.

TAN is an important parameter and affects pH by influencing the buffering capacity of the medium. At high TAN concentration, inversely proportional to increase in buffering capacity the bioleaching efficiency would presumably decrease. In this study, TAN concentration and total alkalinity exceeded 1,350 mg/l and 6,200 mg ($CaCO_3$) /L, respectively at the end of phase II on day 97 (Figures 3.2c and 3.2d).

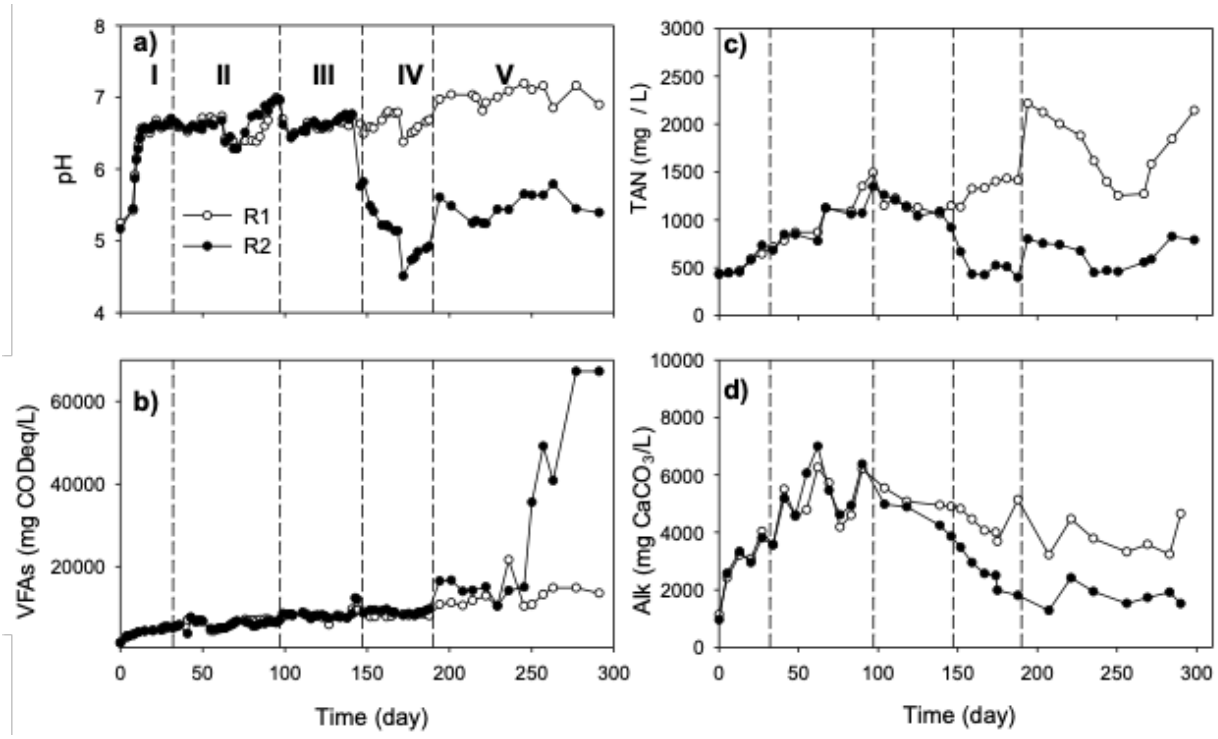

Figure 3. 2. pH (a); volatile fatty acids (b); total ammonia nitrogen (c); total alkalinity (d). Vertical dashed lines indicating the duration of each phase

Increasing OLR to 16.7 gVS/L·d starting from day 98 (phase III), resulted slight increase in VFAs concentration and then plateau was reached at 8550 ± 615 mg CODeq/L in both reactors until day 141. Although it was hypothesized that increasing OLR may stimulate decrease in pH values, because of high TAN concentration (>1150 mg/L) and the corresponding high alkalinity in the reactors (>4200 mg $CaCO_3$/L), the pH did not decrease lower than 6.6 (Figure 3.2a). In R2 and phase IV, the OLR was reduced to 8.91 gVS/L·d and as an additional carbon source, 2mg/L of readily biodegradable organic carbon; i.e., sucrose was added daily into R2. Therefore, TAN and total alkalinity were decreased from 1,090- and 6,900 mg $CaCO_3$/L to 523- and 1980 mg $CaCO_3$/L until day 175, respectively and remained around these values until the end of phase IV. As a

consequence of low TAN concentration and low buffering capacity of R2, pH considerably decreased over time and dropped to 4.5 (Figure 3.2a). In the last phase, operational OLR and SRT were 17.65 gVS/L·d and 4 days, respectively (Figure 2.2). VFA accumulation, over 15,000 mg CODeq/L in R1 and more than 67,200 mg CODeq/L in R2, was observed. Significant VFA accumulation in R2 was observed as a result of quick consumption of readily biodegradable COD in the form of sucrose ($p < 0.05$) (Figure 3.2b). Higher pH levels were observed in R1 compared to that of R2, which might be related to slow biodegradation of WAS as a result of presence of cells, particulate matter, and slowly biodegradable COD. Low pH in R2 compared to that of R1, is probably due to low alkalinity caused by eradication of easily biodegradable organic carbon that generates significant amount of VFAs at high OLR. In R1, alkalinity was in the range of 3,300 to 4,800 mg $CaCO_3$/L, while 50% of this range was determined in R2.

3.2.2. Anaerobic bioleaching of potentially toxic metals during the fermentation process (Acidogenic reactors)

Soluble portion of Cr in both reactors (R1 and R2) was low during the whole fermentation process (Figure 3.3a). Both R1 and R2 showed the same pattern in phases I, II and middle of phase III and the percentage in the soluble fraction was un-detectable (Figure 3.3a). On day 140, tiny enhancement (<7%) in the solubilization of Cr was observed. After that gradual reduction in the solubility of Cr was observed and in the last phase (phase V), maximum concentration of 6% was determined in both R1 and R2 (Figure 3.3a).

In the case of Ni, there was a strong correlation between the drop of pH and Ni solubility. At the beginning of study and when the pH was lower than 6, in average higher than 22% of Ni was observed in both reactors. Inversely proportional to increase in pH values, declining trend in the solubility of Ni was observed and decreased to lower than 5% at the end of phase II on day 97. With increasing OLR to 16.70 gVS/L·d at the beginning of phase III on day 98, slight increasing trend in VFAs concentration accompanied with tiny reduction in pH values, resulted increasing in the soluble fraction of the Ni and reached to 17% in both reactors. In R2 and when the pH declined to 4.5, Ni significantly solubilized and peaked to 30% on day 159 ($p < 0.05$). From day 162 slight decreasing trend in the solubility of Ni was observed and remained around 26 % at the end of phase IV (Figure 3.3b). Yet, the mobilization efficiency of Ni in the control reactor was around 14 %, since the pH remained above 6. Soluble fraction in R1 and R2 was decreased from 14% and

26% to 4.5% and 18.6 % until day 245 and then remained around 8 and 20% until the end of study, respectively (Figure 3.3). Due to high VFAs concentration causing low pH, higher soluble fraction of Ni was determined in R2 (Figure 3.3). Former studies have also reported high percentage of Ni in the unstable fractions such as exchangeable and carbonate bounded fractions, of digestate (Alvarez et al., 2002; Hei et al., 2016). Likewise, Zhang et al. (2016) reported that in anaerobic digestion of sewage sludge, Ni distributed considerably in the mobile fraction and likely released into the water-soluble environment.

The results exhibited that Cu solubilization was much less dependent upon pH and it was the most difficult metal to be solubilized (Figure 3.3c). Even though the pH was brought to 4.5, the leaching efficiency did not improve in R2, indicating a complex origin or form of the Cu in the sludge. Jenkins et al. (1981) showed that Cu existed as an organic complex in the sludge that has strong affinity to the organics presented in the sludge. Moreover, Dong et al. (2013) reported that during anaerobic digestion process, Cu transformed from the most solubilized form to stabilized organic forms. Compared to Cu, zinc (Zn) was the second most difficult metal to be solubilized under anaerobic conditions (Figure 3.3d). Even when the pH of R2 declined to 4.5, there was no significant difference ($p > 0.05$) in the soluble fraction of Zn between two reactors.

The results obtained in this section indicate that accumulation of VFAs that lowered the pH, alkalinity and TAN, enhanced the solubility of Ni, while no significant changes were observed on the solubility of Cu, Cr and Zn ($p > 0.05$). It has been reported that the possibility of the extraction of intracellularly entrapped metals from biomass is low (Papirio et al., 2017) and the extraction can be achieved only by the cell biomass destruction. Therefore, low bioleaching efficiency for Cu and Zn can be described with intracellular accumulation of these metals to the microbial cells. Moreover, presence of nitrogen in amino-sugar and oxygen content of hydroxyl and carboxyl groups within EPS mainly bind Cu and Zn with strong covalent bonds (Fang et al., 2011; Ha et al., 2010), whereas interaction of Ni with EPS is weak (Papirio et al., 2017), indicating low adsorption affinity to the EPS.

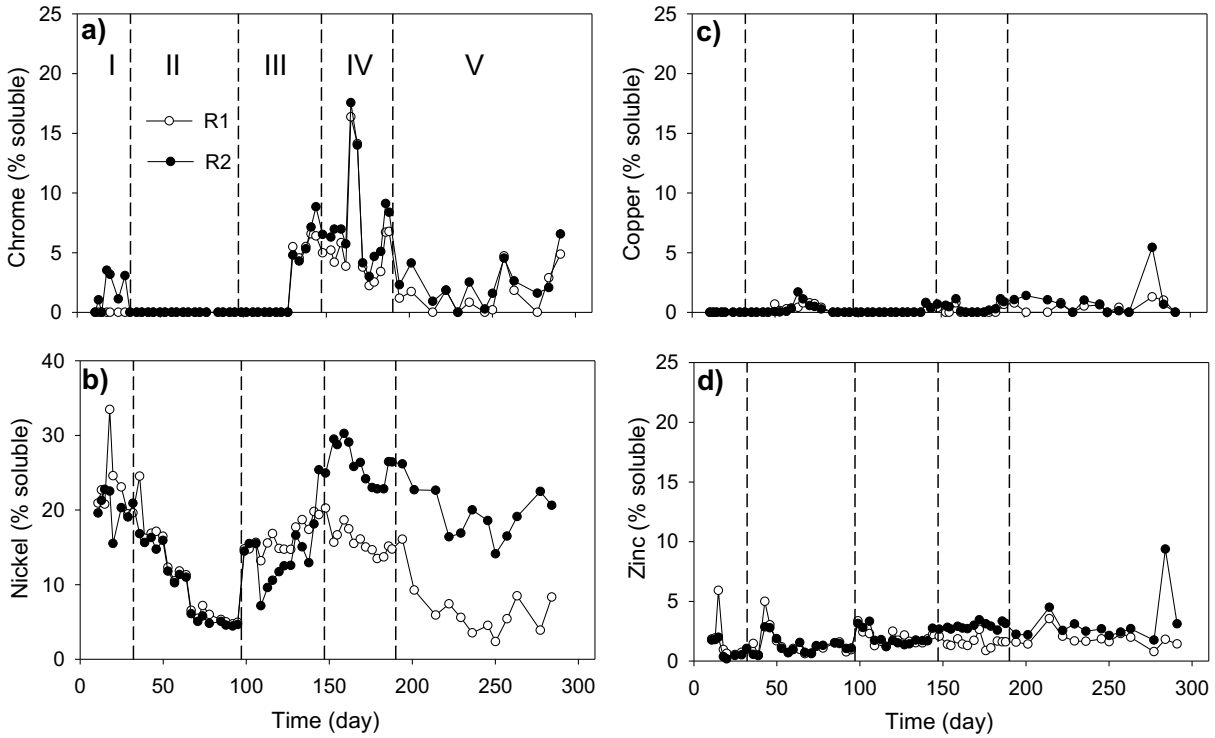

Figure 3. 3. Percentage of heavy metals in liquid fraction of the effluent digestate. Vertical dashed lines indicating the duration of each phase

3.3. Anaerobic Digestion of WAS in Methanogenic Reactors

3.3.1. Performance of the reactors during the digestion process (Methanogenic reactors)

This part of the study was divided into three phases with total duration of 170 days (Figure 2.3). As a result of decrease of OLR from 17.65 gVS/L·d in the previous part of the study (section 3.2) to 2.68 gVS/L·d in phase I of this part of the study, and increase of SRT from 4 to 16 days, fast consumption of VFAs in R1 resulted a CH_4 yield of 0.18 L/gVS·d (Figure 3.4a). From the beginning of phase I to the end of this phase in R2, observation of high VFAs concentration (13,470 ± 1,710 mg CODeq/L) and low pH values (<5.3), indicated low buffering capacity and instability of the reactor (Figure 3.4). As a result of decreased pH levels in R2, CH_4 production of the reactor decreased and perhaps over time methanogens were washed out (Figure 3.4). In this part of the study, R3 was operated with the same operational conditions (OLR of 2.68 gVS/L·d and SRT of 16 days) and thickened WAS from Ataköy WWTP and ultrasound pretreated grass were used as substrates and the average CH_4 yield of 0.19 L/gVS·d was obtained (Figure 3.4a). The pH of the reactor remained around 7.2 (Figure 3.4c). In order to compensate for probable loss

of methanogens due to VFAs accumulation and thus pH decrease in R2, the reactor was bio-augmented using the digestate from R1 for two consecutive days, and ultrasound pretreated grass was added as a co-substrate instead of sugar at the beginning of phase II. Consequently, fast consumption of VFAs and rapid increase in pH values resulted in a sharp increase in CH_4 yield, which exceeded 0.30 L/gVS·d within 10 days (Figure 3.4a). Subsequent to utilization of VFAs, CH_4 yield decreased and remained around 0.17 L/gVS·d at the end of phase II (Figure 3.4a and 3.4b). Towards the last days of phase II, the average CH_4 yield for R2 and R3 was in the range of 0.15 to 0.18 L/gVS·d, whereas, the average CH_4 yield for R1 remained between 0.13 to 0.15 L/gVS·d (Figure 3.4a). In the last phase, OLR was increased to 3.02 gVS/L·d in all reactors. At the beginning of the last phase, CH_4 yield in R1 dropped to 0.05 L/gVS·d and then recovered again and reached to 0.11 ± 0.01 L/gVS·d (Figure 3.4a). However, as a result of increase of OLR on 140th day of the operation, CH_4 yield gradually decreased and reached to 0.10 L/gVS·d towards to end of the study (Figure 3.4a). Increase of OLR perhaps resulted in the increase of extracellular polymeric substances (EPS) and/or inorganic substances in all reactors. The accumulation of EPS and substantial organic-bound metals within it may bridge the cells and protect them from biological eradication and thus generating low biogas production from conversion of WAS (Xu et al., 2017). Low CH_4 yield towards the end of the study can also be correlated to high concentration of Cr in the soluble fraction that exceeded the inhibitory level, which has also mentioned in the study of Demirel and Yenigün, (2002).

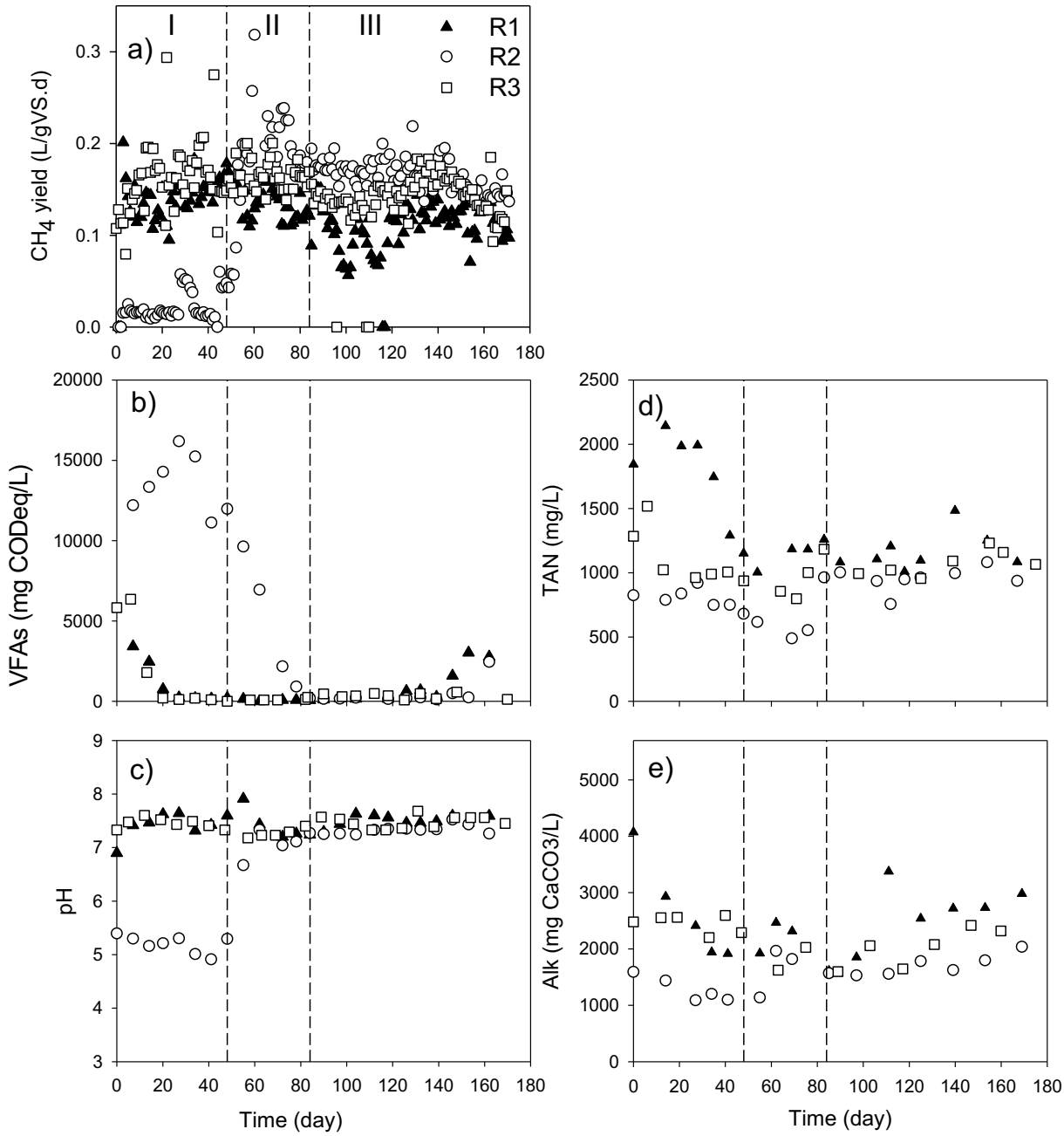

Figure 3. 4. CH_4 yield (a); volatile fatty acids (VFAs) (b); pH values (c); total ammonia nitrogen (TAN) (d); total alkalinity (e). Phase I: (0-32 days); Phase II: (33 - 97 days); Phase III: (98 - 147 days); Phase IV: (148 - 190 days); Phase V: (191- 298 days)

3.3.2. Anaerobic bioleaching of potentially toxic metals during the digestion process (Methanogenic reactors)

Anaerobic bioleaching experiments were conducted in three reactors and change of operational conditions were investigated in three phases (Figure 2.3). Gradual decrease in soluble portion of Cr for all reactors was observed and lower than 5% for R1 and R2 and lower than 8% were determined in R3 at the end of phase I (Figure 3.5). In phase II, feeding regimes in R1 and R2 were changed and in R3 feeding regime remained the same (Figure 2.3). Interestingly, in R1, soluble portion of Cr remained un-changed, whereas in R2 and R3 increased tendency in the solubility of Cr was observed (Figure 3.5). Soluble fraction of Cr in R1 reached to only 10% at the end of phase III, while the same fraction in R2 and R3 was continued to increase linearly and reached to 25% and 20% at the end of the study, respectively (Figure 3.5). The results of this study revealed that even when the VFA concentration exceeded 16,000 mg CODeq/L during phase I, maximum solubility of Cr was 6.6% (Figures 3.4b and 3.5a). Therefore, in this study rather than VFAs, complexation of Cr with another ligand; i.e., DOM, might have potentially increased its concentration in the liquid fraction. Adsorption between metals and the solid components of digestate, and complexation between metals and soluble organic matter of digestate co-exist during the digestion process. At low pH, DOM have low solubility and thus soluble fraction of Cr at higher pH is the indication of forming complexation with DOM (Borggaard et al., 2019). Therefore, Cr solubility appeared to increase in response to decreased acidity. The results of this study are in good agreement with the study of Xie et al. (2015), which reported decline of Cr solubility in response to the increased acidity. Similarly, Gustafsson et al. (2014) showed a pattern of Cr dissolution as a function of pH in the soil suspension that supports our results in this study. Although all the reactors were operated with the same OLRs, due to co-substrate addition, R2 and R3 were loaded with lower WAS compared to that of R1. Under these operational conditions, biodegradation efficiency of WAS in R1 was recorded as 6.5%, while higher biodegradation efficiencies (~22.7%) were obtained in R2 and R3. Increase of biodegradation efficiency indicates increase of dissolved organic matter, which presumably enhances Cr solubilization through ligand effect. Therefore, extraction of metals complexed with particulate organic matter was perhaps limited under anaerobic conditions due to lower biodegradation efficiency at higher WAS concentration.

At the beginning of this part of the study, Ni concentration was more than 30% in the soluble fraction of R2 (Figure 3.5b). Although no considerable changes in pH values were observed with respect to the first part of the study, high soluble fraction of Ni was perhaps due to abrupt decrease of OLR from 17.65 gVS/L·d in the previous section (section 3.2) to 2.68 gVS/L·d in this part of the study. With the progress of AD process, sharp decreasing trend in Ni solubilization of R2 was observed and reduced to values lower than 20% (Figure 3.5b). Due to high pH values, soluble fraction of Ni was lower than 10% in R1 and declined to lower than 2% in R3 in phase I (Figure 3.5b). In phase II, slight variation in Ni solubility of R1 and R3 was observed. Soluble fraction of Ni decreased to lower than 2% in R1 and increased to 10% in R3 (Figure 3.5b). In R2, fast decline in soluble fraction of Ni was recorded in Phase II (Figure 3.5b). Decreasing trend can be linked to the sharp increasing of pH in R2 due to bioaugmentation of this reactor (Figure 3.4c and 3.5b). In phase III, soluble fraction of Ni in all reactors was lower than 10% (Figure 3.4b). In this study, Ni solubility decreased when the pH values increased and hence, Ni solubility appeared to decline in response to the decreased acidity. Accordingly, Fang et al. (2016) reported that as the pH increased, declining trend in anaerobic bioleaching of HMs from soil amended with sewage sludge was observed.

In phase I, soluble fraction of Cu in average was lower than 5% in both R1 and R2. Maximum solubilization efficiency was 8% in R3 (Figure 3.5c). In phase II, no discernible improvements on the soluble portion of Cu were observed in the reactors studied. Solubility of Cu in R3 was higher than that of R1 and R2 and reached to 10% at the end of phase II (Figure 3.5c). However, in the last phase, soluble portion of Cu in R2 was lower than 5%, insignificant enhancement in soluble fraction of R1 and R3 was observed that reached to 9 and 15% of the total concentration, respectively (Figure 3.5c). Higher soluble portion of Cu in R1 and R3 compared to that of R2 might be the results of different WAS that was fed into the reactors. It is evident that distribution of Cu in organic bound fraction of thickened WAS from Ataköy WWTP is higher with respect to dried WAS from Paşaköy WWTP (Figure 3.1). As a consequence of organic matter degradation, Cu exhibited relatively high release ability in R1 and R3 in the last phase (Figure 3.5c). Low solubility of Cu in this study can be explained by strong affinity and re-complexation with organic matter during the digestion process that limits its solubilization. Accordingly, Sheng et al. (2013) reported that binding enthalpy between EPS of sludge and Cu^{2+} is exothermic and protein within

the EPS can be strong ligands for Cu^{2+}. Moreover, Couillard and Mercier. (1990) highlighted that rise in oxidation reduction potential (ORP) is the key parameter for Cu solubilization, and the ORP values greater than +250 mV is required for Cu solubilization. However, considering that ORP values in the AD process is lower than -300 mV, Cu solubilization becomes challenging.

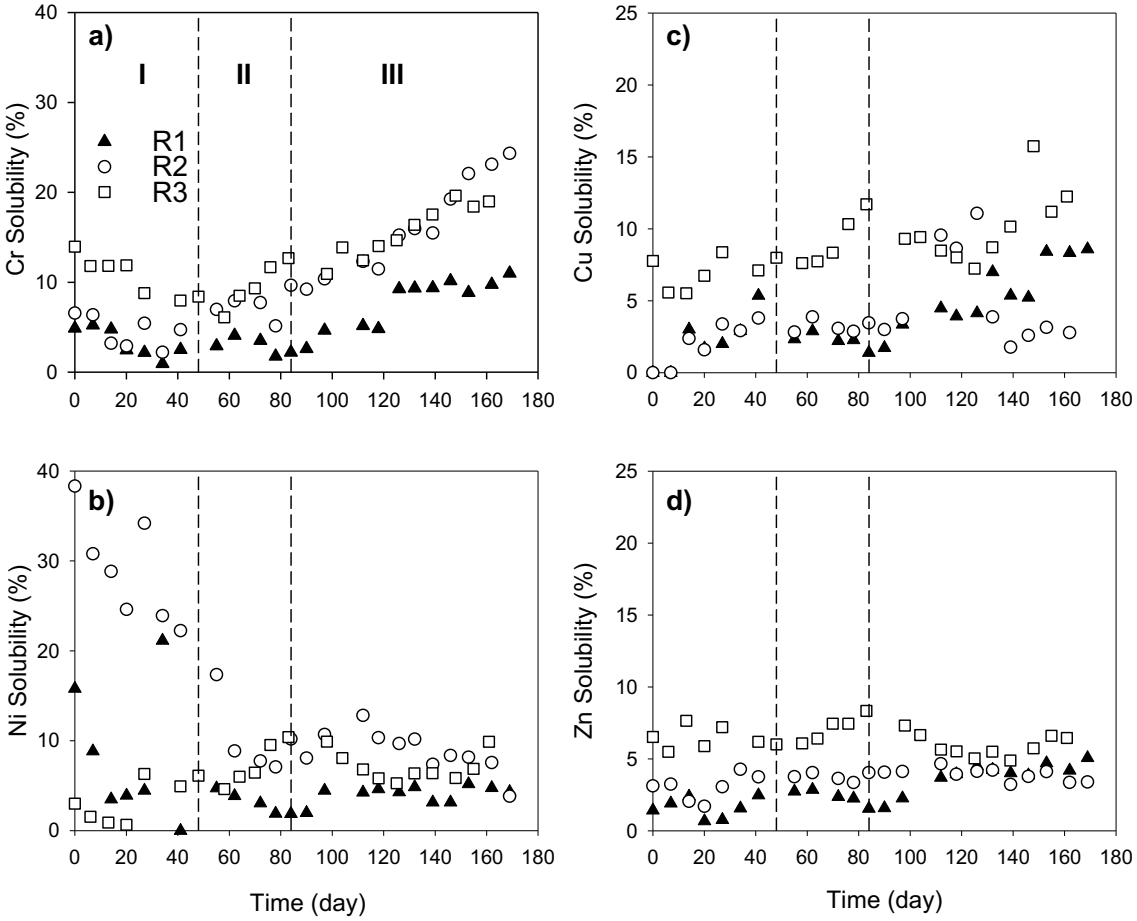

Figure 3. 5. Percent soluble of potentially toxic metals in the effluent digestate. Vertical dashed lines indicating the duration of each phase

Decrease of OLR from 17.65 gVS/L·d in the previous section of the study (section 3.2) to 2.68 gVS/L·d in this part of the study, no obvious changes in the soluble fraction of Zn was observed (Figure 3.5d). In phases II and III, soluble portion of Zn remained consistently low, and never exceeded 5% of the total concentration in both R1 and R2 (Figure 3.5d). In phases I and II, partitioning of Zn in the soluble fraction of R3 remained around 7% that was nearly two times

higher compared to that of the same fraction of other reactors (Figure 3.5d). In the last phase, soluble portion of Zn decreased and in average 5.6% ± 0.6 of the total concentration was determined (Figure 3.5d).

At the end of the study, there was small variation in the soluble fraction of all reactors (Figure 3.5d). Likewise, in the study of Dong et al. (2013) low solubility of Zn was determined in WAS during AD and with the progress of the digestion process the solubility was decreased to less than 2%. In phases I, II and beginning of phase III of R3, high solubility of Zn with respect to other reactors, might be attributed to the origin of WAS. Distribution of Zn in the organic bound fraction of thickened WAS from Ataköy WWTP was higher than the dried WAS from Paşaköy WWTP (Figure 3.1). Degradation of organic matter perhaps resulted in release of organically-bound fraction, resulting relatively higher soluble Zn percentage in R3. However, R1 was fed with thickened WAS, due to high WAS load, organic matter that remained without degradation may have adsorbed Zn and limit the solubilization efficiency. The low efficiency of soluble fraction might be linked to the limited solubility of relevant metal salts. At higher WAS concentration, particulate organic matter that is not hydrolyzed can retain adsorbed or complexed metals.

3.4. Migration Behavior of Potentially Toxic Metals During the Digestion Process

The solubilization efficiency in the progress of AD might be predicted from the speciation of target metals. Therefore, differences in metal leaching efficiency might be explained via fractionation of metals during the digestion process. In this part of the study, samples from the last phase of the anaerobic fermentation process (acidogenic reactors) (section 3.2) and samples from all phases of anaerobic digestion process (methanogenic reactors) (section 3.3) were selected for determining fractions of HMs in the effluent digestate of all reactors. In R1, regarding Cr, the dominant binding species was the residual fraction in phase V of the first part of the study (Figure 2.2) with 59.2% of the total concentration, followed by organic bound, exchangeable and carbonate bound fractions with 29%, 8.6% and 3.2%, respectively (Figure 3.6). In the second part of the study (Figure 2.3); in which SRT increased to 16 days, and in phase I, small variation in the distribution of HMs was determined. Compared to previous section (Section 3.3), almost 2% decrease in residual fraction of Cr was accompanied with small enhancement in the organic bound fraction (Figure 3.6a). In

phase II, organic bound fraction elevated to 39.9% and the residual fraction of Cr decreased to 48.3%. Moreover, small increment (1.1%) in the carbonate fraction accompanied with small decrease in exchangeable fraction to 6.7% (Figure 3.6a). Deviation in the distribution of HMs in phase II, can be explained by changes in the feeding regime of the reactor where the reactor was started to be fed with thickened WAS from Ataköy WWTP instead of dried WAS from Paşaköy WWTP (Figure 2.2). As it is evident percent distribution of HMs in the organic bound and carbonate fractions of thickened WAS from Ataköy WWTP was higher with respect to dried WAS from Paşaköy WWTP (Figure 3.1). In phase III small changes in the distribution of HMs were observed and 5% increment in residual fraction and small decrease in the organic bound and exchangeable fractions were observed (Figure 3.6). In R2, the residual fraction of Cr in phases IV of the first part of the study and phase I of the second part of the study were similar to the values observed for R1 (Figure 3.6). The exchangeable fraction of Cr in R2 was higher and the values of 15 and 11% were recorded in phases V of the anaerobic fermentation process (section 3.2) and phase 1 of the anaerobic digestion process (section 3.3), respectively (Figure 3.6b). Compared to R1, higher exchangeable fraction of Cr might be the results of readily biodegradable organic carbon addition into R2 (Figures 2.2 and 2.3). The exchangeable fraction of Cr in R2 increased further and reached to 15.6 and 23.5% in phases II and III, respectively. In the same reactor, small increment in organic bound fraction of Cr was observed in phase II, while declining trend was seen in phase III. Residual fraction of Cr decreased to 43.4% in phase II and remained around this value in the last phase (Figure 3.6b). In R3, the exchangeable fraction of Cr increased from 4.3% in phase I to 13.1% in phase II and reached to 26% of the total concentration in the last phase (Figure 3.6c). These results are in good agreement with the bioleaching processes (Figure 3.5a) in which 25% and 20% of the total concentration of Cr was leached in both R1 and R2, respectively as the pH increased to neutral level. Increase Cr solubility with decrease acidity has been reported previously in the study of Xie et al. (2015). Carbonate fraction remained with negligible changes from phase I to II and slightly increased to 4% in the last phase in R3. Organic bound fraction of Cr increased from 26.7% in phase I to 36.6% in phase II and dropped to 25.5% in the last phase in R3 (Figure 3.6c). Residual fraction of Cr was 66% in phase I, which gradually decreased to 47% in phase II then to 44.6% in phase III in R3 (Figure 3.6c). Although, R2 and R3 were fed with different WAS, distribution of Cr, in particular exchangeable fraction, followed the same trend that differed from R1 (Figure 3.6). Therefore, origin of WAS might also affect the speciation of

metals since speciation of Cr in R2 and R3, where the WAS from same source was used, were similar, whereas speciation in R1 was different from the other two.

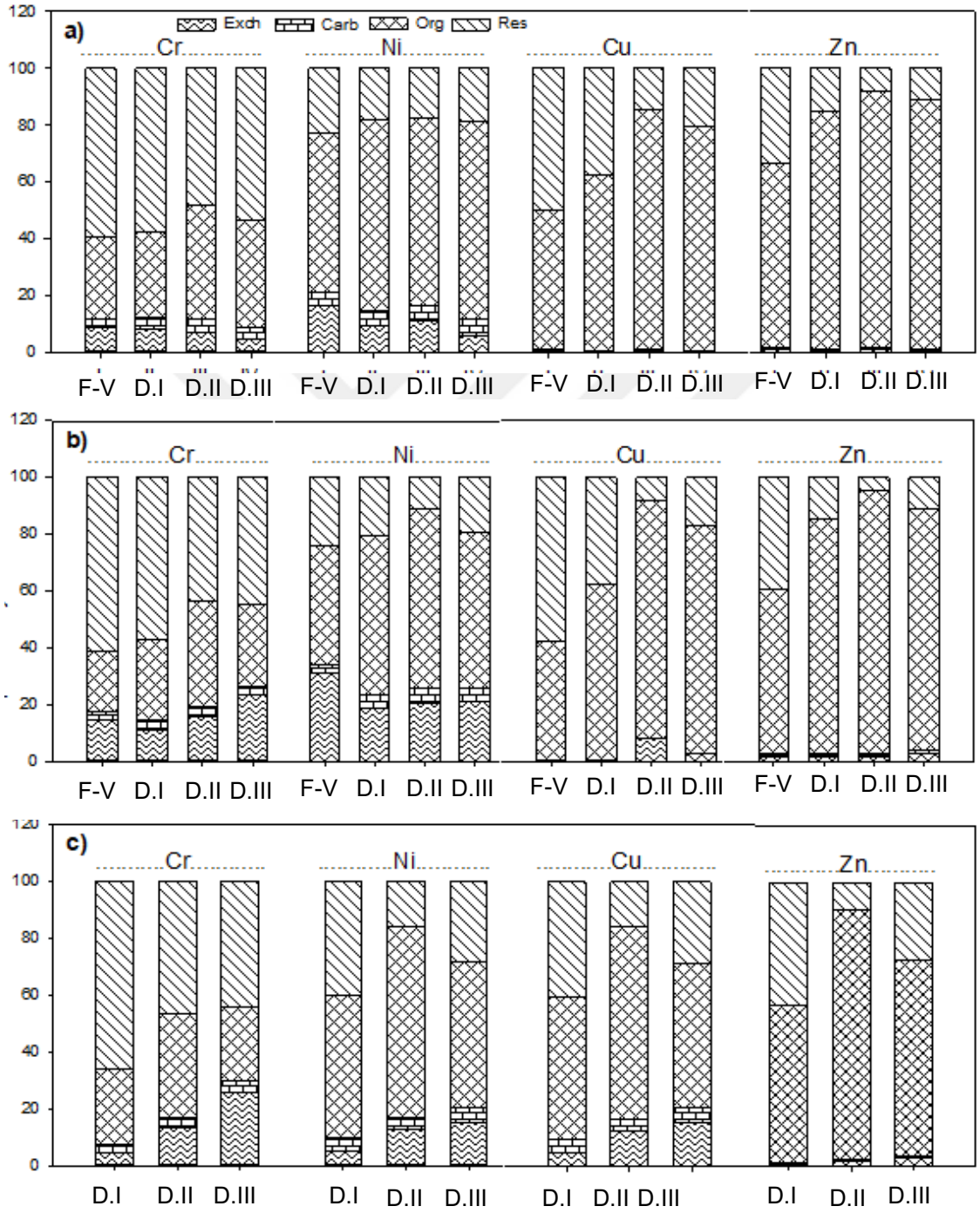

Figure 3. 6. Fractionation of metals from the effluent of digestate; reactor 1 (a), reactor 2 (b), reactor 3 (c). F.V: represents sample of section 3.2, phase V; DI: represents sample of section 3.3, phase I; DII: represent sample of section 3.3, phase II; D.III: represent sample of section 3.3, phase III

Regarding Ni; in R1 from phase V of the anaerobic fermentation process (section 3.2) to phase I of the anaerobic digestion processes (section 3.3), 7% decrease in the exchangeable fraction, coincided with increasing in organic bound fraction that reached to 67% and decrease of residual fraction that reduced to 18%. In phase II, organically bound fraction decreased slightly to 66%, while tiny increment in the exchangeable fraction of Ni from 9.2 to 11% was observed. In phase III, organically bound fraction increased to 69.2%, whereas exchangeable fraction was decreased to 5.9%. Presence of Ni in the carbonate fraction was almost stable (Figure 3.6a). In R2, Ni was highly distributed between exchangeable, organic bound, residual and carbonate fractions of the effluent digestate. In phase V of the anaerobic fermentation process (section 3.2), approximately 31% of Ni was detected in the exchangeable fraction, which can be correlated to the lower pH of R2 (Figure 3.6b and 3.6b). In phase I, exchangeable fraction of Ni decreased to 19%. By contrast, organic bound fraction increased from 41.5% in phase V of the anaerobic fermentation process (section 3.2) to 56% in phase I. In phase II, 9% decrease in residual fraction of Ni occurred with 7.2% increment in the organic bound fraction (Figure 3.6b). In the last phase, fractionation of Ni was almost similar to the distribution in phase I (Figure 3.6b). Exchangeable fraction of Ni in R3 increased from 4.8% in phase I to 12.6% in phase II and exceeded 15 % in phase III (Figure 3.6c). These results are consistent with the study of Suanon et al. (2016) in which 11.2% of Ni was determined in soluble fraction of sludge anaerobic digester. In phase II, significant decrease in residual fraction of Ni was observed, while organic bound fraction reached to 67.6%. Increase in residual fraction of Ni from 16% in phase II to 28.5% in phase III, coincided with decrease of organically bounded fraction of Ni from 67.6% in phase II to 51% in phase III (Figure 3.6c). Speciation of Ni in R2 and R3 was expected to follow the same trend; however, in R2 exchangeable fraction was higher than R3. Exchangeable fraction of Ni in dried WAS from Paşaköy WWTP, was higher than that of thickened WAS from Ataköy WWTP (Figure 3.1). Therefore, this deviation can be described by the characteristics of two different WAS in terms of Ni distribution in different fraction.

AD altered Cu speciation mostly between organically bound and residual fractions of all reactors. In R1, almost 99% of the Cu was determined in these two fractions (Figure 3.6a). From phase V of the anaerobic fermentation process (section 3.2) to phase I of the anaerobic digestion process (section 3.3), the organic bound fraction was increased from 48.7 to 61.6% (Figure 3.6a). The

organically bound fraction of Cu continued to increase and reached to 84% in phase II (Figure 3.6a). In the last phase 78.6% of the total concentration of Cu was found in the organically bound fraction (Figure 3.6a). In all phases of R1, residual fraction of Cu changed inversely proportional to its organically bound fraction (Figure 3.6a). Likewise, in R2, Cu was dominantly distributed between organic bound and residual fractions. In phases II and III, 8 and 3% of the total concentrations were determined in the exchangeable fraction, respectively (Figure 3.6b). In R3, sum of organically bound and residual fractions exceeded 98, 88 and 93% of the total concentration in phases I, II and III, respectively (Figure 3.6c). In phases II and III more than 10 and 6% of Cu was determined in exchangeable fraction. Generally, Cu is known to be dominantly associated with the organically bound fraction (Dąbrowska, 2012). Likewise, our results verified this argument as considerable portion of total Cu was measured in the organic bound fraction. Moreover, high partitioning of Cu in the residual fraction indicated that Cu was included in minerals existed in the digestate. Walter et al. (2006) also reported the existence of over 20% of Cu in residual fraction of sludge from different treatment plants. Although Cu is distributed mostly between two fractions of the digestate in all reactors, the exchangeable fraction of Cu in R2 and R3 was relatively higher than that of R1 (Figure 6). The amount of WAS that fed in to R2 and R3 was lower than R1, therefore, higher exchangeable fraction of Cu in these reactors might be attributed to their different feeding regimes (Figure 2.2). Accordingly, Villar and Garcia, (2002) highlighted that low TS content of the WAS can be decomposed rapidly and may increase the release of the organically bound fraction of Cu.

In this study, sequential extraction procedure for metal fractionation revealed that, Zn exist predominantly as organically bound, followed by residual fraction in the digested samples of all reactors (Figure 3.6). The availability of Zn in other fractions was low (<3%) (Figure3.6). From phase V of the anaerobic fermentation process (section 3.2) to phase I of the anaerobic digestion process (section 3.3), the proportion of organic bound fraction increased to levels greater than 82% in both R1 and R2 (Figure 3.6a and 3.6b). While residual fraction of Zn decreased to less than 15% in R1 and R2. In phase I of R3, 55.3% and 43.5% of the total concentration were detected in organically bound and residual fractions, respectively (Figure 3.6). In phase II, organically bound fraction of Zn exceeded 90% in R1 and R2 and 88% in R3. The abundance of Zn in residual fraction decreased to lower than 8% in R1 and R2 and less than 10% in R3 (Figure 3.6). In the last

phase small variation in the fractionation of Zn in R1 was observed, while proportion of the residual fraction of Zn in R2 and R3 were elevated to 11 and 27%, respectively. Moreover, small increase tendency in the exchangeable fraction of R3 was observed and 3.3% of the total concentration was detected in this fraction. Results of this study are consistent to a certain extent with the findings of Zhang et al. (2016) in which 60% of Zn in the total concentration was determined in organic bound fraction. High presence of Zn in the organically bound fraction, might be explained by adsorption of Zn to EPS with strong covalent bound that form with inner sphere complexes (Ha et al., 2010). Results of former studies concerning influence of AD on Zn speciation were not consistent. Dong et al. (2013) highlighted that AD has no influence on Zn speciation in sewage sludge, while other studies indicated increasing tendency of the organic bound fraction during the digestion process (Dąbrowska and Rosińska, 2012; Legros et al., 2017; Marcato et al., 2009).

To sum up, our results revealed that HMs were dominantly bound to organics or were in the residual fractions of digestate in all phases of the anaerobic digestion process (Figure 3.6). Transformation of HMs were affected by the presence of high molecular weight hydrophobic EPS. Metal ions mainly existed as cations, which increased the complexation ability to organic matters. Based on the divalent cation binding theory, functional groups such as carboxyl, hydroxyl, etc. embedded in EPS, can form a bridge between organic compounds and metals (Xu et al., 2019). Moreover, low VS/TS ratio in both dried WAS from Paşaköy WWTP and thickened WAS from Ataköy WWTP can be related to high inorganic content of the WAS (Table 2.1) that indicates the presence of metals in the crystalline structure of the solids.

3.5. Application of Advanced Oxidation Processes Using Fenton Reaction as a Post-treatment Tools for Solubilization of HMs from the Solid State of the Digestate

Advanced oxidation technologies, e.g., Fenton oxidation, sulfate radical oxidation, microwave-H_2O_2 oxidation processes, and permanganate/bisulfite electro dewatering have been investigated and proven to be effective processes for the solubilization of sludge (Xu et al., 2019). It has been reported that under wet oxidation processes, more than 70% of the biopolymers can be decomposed (Urrea et al., 2017) and HMs may migrate and transform into the mobile fractions.

In this study, chemical post-treatment of the digestate was applied to break-down the complex structure of cell walls and EPS in digestate flocs and to enhance HMs removal efficiency from the solid state of the digestate. In this regard, to demetallize digestate, in this part of the study, as a post-treatment process, Fenton's reagents (Fe^{+2} + H_2O_2) and H_2O_2 solely (Figure 3.7) as strong oxidants, were used at pH: 2 and pH: 7 by adjusting the pH using HCl and NaOH. The results revealed that at neutral pH; using H_2O_2 and mixture of H_2O_2 with Fe^{+2}, no improvement in the solubility of HMs was observed (Figure 3.7). Using Fenton's reagent, Neutral pH adversely affected the solubilization and it was declined to almost zero for all metals. This can be explained by precipitation of Fe^{+2} and HM at neutral pH (Yu et al., 2016). Therefore, while using Fenton's reaction as a post-treatment tool, acidic environment is required to prevent the precipitation of iron and other HMs (Yu et al., 2016). With decreasing the pH to 2 using HCl, significant improvement in the solubility of Ni and Zn was observed ($p < 0.05$) and their concentrations in the soluble fraction increased to 55% and 45%, respectively. High solubility of Ni and Zn at low pH can be linked to the increased cations competition for binding sites and minor biosorption efficiency (Papirio et al., 2017). On the other hand, no significant changes in the solubilities of Cu and Cr were observed ($p>0.05$) (Figure 3.7). Low solubility of Cu can be described by the strong binding capacity of the Cu to the COOH groups, phosphoryl groups and amino functional groups available in the cell structure of the microorganisms. Moreover, living cells can uptake Cu through intracellular accumulation (Golab et al., 1995). Low solubility of Cr can be correlated to the high partioning of the Cr in the residual fraction of the digestate. Moreover, low solubility of the Cr can be linked to the high biosorption of Cr that has superior interaction of metal with biomass under acidic conditions (Srinath et al., 2002; Velásquez and Dussan, 2009). Compared to acidic level (pH:2), application of H_2O_2 at the same pH (pH:2), slightly enhanced the solubilization of Ni and Zn which increased to 71% and 61% of the their total concentration, respectively. However, significant improvement in the soluble form of Cu was observed ($p<0.05$) and from almost non-soluble form reached to almost 45% of the total concentration. It can be deduced that, interacted portion of the Cu with carboxyl and phosphoryl functional groups on the microbial cell surface might be broken-down using H_2O_2 at acidic conditions.

Post-treatment of the digestate using H_2O_2 at acidic level and Fenton's reagent (H_2O_2/Fe^{+2}) at the same pH level resulted in almost same solubility for Ni, Cr and Zn.

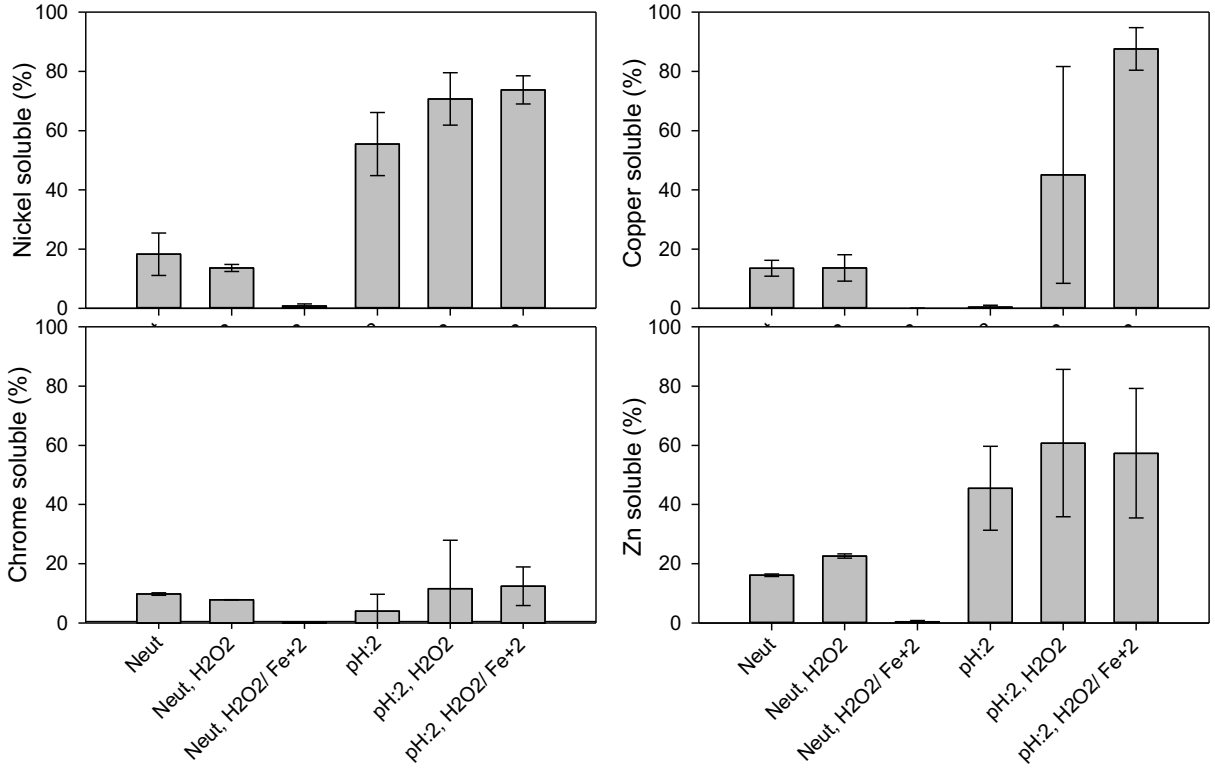

Figure 3. 7. Influence of acid, H_2O_2, and Fenton's reagent (H_2O_2/Fe^{+2}) on the solubility of HMs from digestate

However, with the application of Fenton reaction (H_2O_2/Fe^{+2}), remarkable improvement in the solubility of Cu was observed and reached to 88% of the total concentration. Cu is intracellularly entrapped (Papirio et al., 2017; Wong et al., 1993) which cannot be extracted, unless microbial cells are disrupted. Generated hydroxyl radical by the mixture of ferrous iron and hydrogen peroxide can react non-selectively with most organic compounds and break-down the rigid structure of the cell wall. Therefore, high Cu leachability can be correlated to the disruption of microbial cells with Fenton's reagent at low pH values.

3.6. Application of Air-assisted Ultrasonication as a Post-treatment Tools for Solubilization of HMs from the Solid Part of the Effluent Digestate

In this study, integration of ultrasonication with aeration was used as a post-treatment tool to enhance the dissolution of HMs from the solid state of the digestate. Solubilization of HMs depend mostly on their chemical forms embedded in sewage sludge. Therefore, metals release efficiency from sewage sludge is restricted due to presence of large number of carboxylic groups in the EPS structure. Moreover, existence of the metals in microbial cell surface is correlated to the interaction

of metals with phosphoryl (Mullen et al., 1989) and amine groups embedded in EPS (Kang et al., 2007). Metals interaction with amine and sulfhydryl functional groups within EPS will strengthen their absorbance onto cell wall and their intracellular accumulation. Thus, extraction of metals from biomass is possible by disruption of microbial cells (Costley and Wallis, 2001; Wong et al., 1993). Post-treatment of the digestate using air-assisted ultrasonication in this study was applied to disrupt the EPS content and surface structure of the particles within the digestate (Figure 3.8).

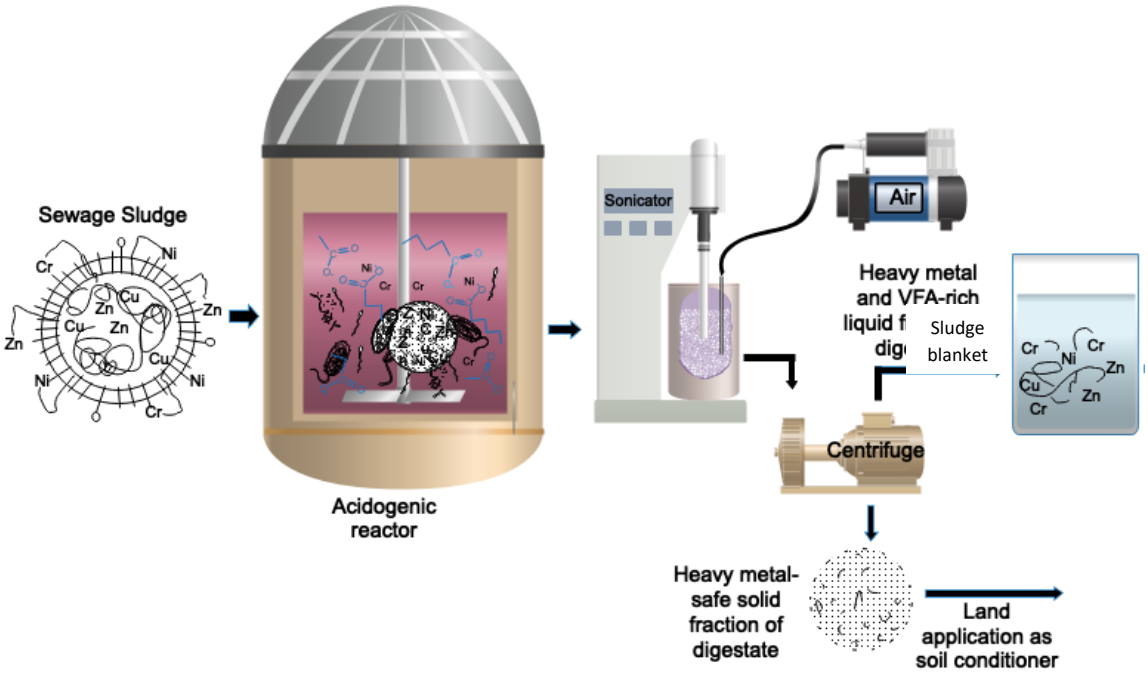

Figure 3. 11. Post-treatment of the effluent digestate and valorization of the subsequent effluents

In this study, metal solubilization was a frequently used parameter to evaluate the efficiency of air-assisted ultrasonication assisted aeration treatment. Post-treatment was applied to the effluent digestate of the acidogenic reactors and the results of HMs solubilization reflected in Figure 3.9. Application of this method, to the digestate significantly increased the solubilization of metals in the samples from reactors (Figure 3.9).

In the sample from R1 and phase I (section 3.2), more than 80% of Ni, 82% of Cu and 74% of Zn were determined in the soluble fraction. Similarly, in R2, more than 80%, 93% and 80% of the total concentrations of Ni, Cu and Zn were determined in the soluble fraction, respectively (Figure 3.9). In phase II, compared to phase I, decrease in the solubilization of metals in both reactors was

observed. Concerning Ni, solubility declined to 77% and 70% in R1 and R2, respectively. In the case of Cu, declining trend was more evident and the solubility decreased from 82% and 93% to 67% and 77% in R1 and R2, respectively (Figure 3.9). In this phase, among the studied metals, the highest decreasing trend in the soluble fraction of Zn was observed and the solubility decreased to 47.5% and 60%, in digestate samples from R1 and R2, respectively. These variations in different phases, were correlated to the different OLRs that were applied to the reactors. As the OLRs increases, the TS and VS inside the reactors would increase as well that decrease the specific energy input to the sample. In phase III, slight decrease in partitioning of Ni in the soluble fraction of samples from both R1 and R2 was observed and the solubility declined to 69% and 66% in R1 and R2, respectively. Likewise, decreasing trend in the soluble fraction of Cu and Zn was observed. In R1, soluble fraction of Cu and Zn decreased to 53% and 38%, respectively. Similar results were obtained in the samples from R2 and the solubility of Cu and Zn declined to 52% and 34.5%, respectively. Compared to phase I, in phases II and III, declining trend in the soluble fractions of metals can be correlated to the high OLRs; and hence, high TS load that were applied to both reactors in these phases (Figure 3.2 and 3.3). In phase IV and in R1, compared to phase III, no obvious changes in the soluble fractions of all metals were observed. This can be described by almost equal specific energy input to the samples taken from R1 in phases III and IV (Table 3.2). By contrast, in R2, soluble fractions of studied metals considerably increased. Partitioning of Ni in the soluble fraction was increased to the maximum value of 85%. Concentration of the Cu and Zn reached to 91% and 73%, respectively. In R2, as a consequence of decrease of OLR on 148th day of the operation, towards to end of the study (Figure 2.2) resulted in the decrease of organic- and/or inorganic substances in R2. In this study, increase in the solubilization of metals was strongly correlated to the specific energy inputs to the samples. In phase IV of the acidogenic reactors (section 3.2) as the VS content in R2 decreased due to low applied OLR, the specific energy inputs of the air-assisted ultrasonication to the samples increased (Table 3.2). With high specific energy input, disintegration of organic flocs via air-assisted ultrasonication, enhanced the metals release efficiency. It has been argued that disintegration of cell membrane starts when energy input exceeds 30 kJ/g TSS, in our case, assisting ultrasonication with aeration may have facilitated the membrane damage while disrupting the organic flocs in the digestate with one third of the energy inputs given in the study of Foladori et al. (2010). Post-treatment results for the solubilization of metals in the samples of acidogenic reactors (section 3.2), taken from phase IV

of R1 and R2, revealed that air-assisted ultrasonication was less efficient in R1, due to high OLR and hence high VS and TS load compared to R2. Digestate involved considerable amount of EPS and substantial organic-bound metals within it may strengthen the stability structure of the flocs (Xu et al., 2017). Therefore, low solubility of metals in samples from R1 was attributed to the low specific energy inputs that lowered the destruction of the digestate particles compared to R2 (Table 3.2).

Table 3. 2. Specific energy input of air-assisted ultrasonication (kJ/gTS)

Phases	Control Reactor (R1)	Test Reactor (R2)
Phase I	9.32	9.42
Phase II	7.07	6.58
Phase III	7.72	6.77
Phase IV	6.98	9.34

To sum up, post-treatment produces a leachate (liquid fraction of digestate) containing high soluble metal concentration and solid fraction of the digestate with low metal content. After solid-liquid separation, HMs can be removed from the liquid fraction of the digestate, e.g., chemical precipitation, adsorption/biosorption, ion exchange, membrane technology, etc. (Yesil and Tugtas, 2019). The subsequent effluent having high VFAs, and low HMs can be utilized as feed-stock in methanogenic up-flow anaerobic reactor. The digestate with low metals content can be recovered as an organic fertilizer in land application. Most agricultural lands in Turkey are organic matter deprived, and hence application of stabilized sludge and/or digestate to agricultural lands would enhance the soil texture and increase the water holding capacity of soil. Numerous studies emphasized that stabilized WAS-amended soils had improved physical, chemical and biological properties, yielding enhanced crop productivity while avoiding chemical fertilizer utilization. Especially, for the countries applying intensive farming on semi-dry lands, utilization of HM-safe digestate can provide additional economic return. The total energy required of the processes proposed in this study (anaerobic digestion + Post-treatment) was 28% higher compared to the only anaerobic

digestion processes. Considering the benefits regarding the utilization of waste-derived organic fertilizers, such as anaerobic digestate, can offset the cost related to post-treatment process used in this study.

3.6.1. Mechanism process of air-assisted ultrasonication

Removal of HMs from sewage sludge involved in three main consecutive steps: an extraction process, solid-liquid separation and finally HM removal followed by recycling the solution to prevent environmental impacts. These three steps are mandatory regardless of the application of the methods used for HM removal from sewage sludge. Among those, the first step is the most important to reveal the extraction efficiency and thus determine the feasibility of the method to be used for HMs removal.

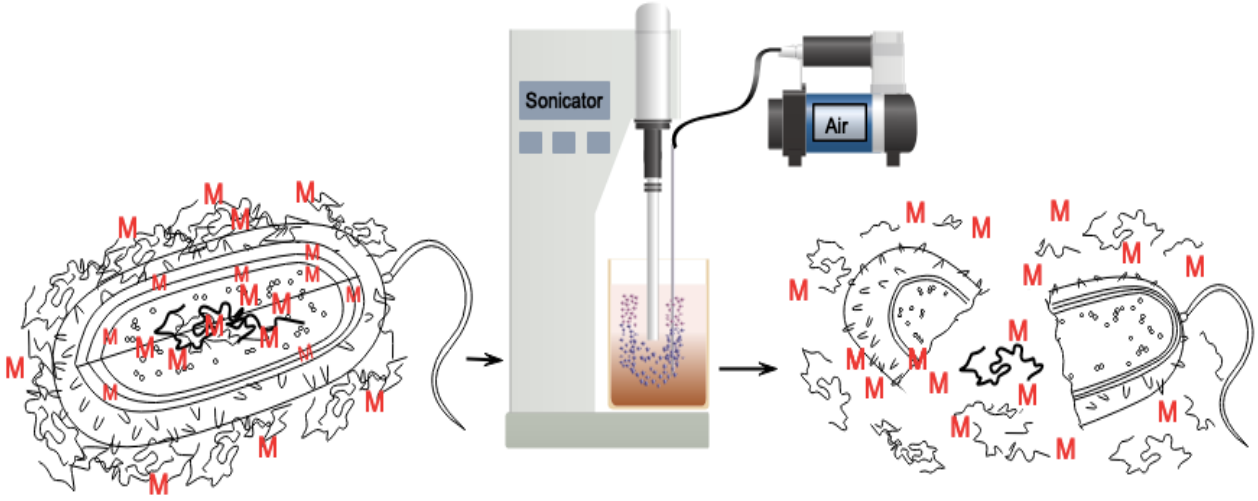

Figure 3. 12. Heavy metal extraction from organic flocs using air-assisted ultrasonication

Acoustic cavitation assisted with aeration and the process of releasing HMs is illustrated in Figure 3.9. Cavitation effects are highly produced by bubbles generated by low frequency ultrasonication of liquid phase, disintegrating the structure of organic matters presented in the digestate and cell walls of the microorganisms. The quick collapse of these cavitation bubbles generates high temperature, pressure and shear stress, which damage the organic flocs. Many studies have found that ultrasonication disintegrates the structure of sludge flocs and cell walls and release intracellular matters into the liquid phase (Chu et al., 2001; Feng et al., 2009; Wang et al., 2006). Aeration accompanies the ultrasonic irradiation, further strengthens HMs release by disintegrating the structure of organic matters presented in the digestate and cell walls of the microorganism.

Shell of supercritical water that surrounds the generated bubbles on the point of crumbling and presence of excess oxygen while aerating the samples, could enhance the generation of OH• radicals in this shell. Additional OH• could also be generated from the destruction of organic substances within the bubbles.

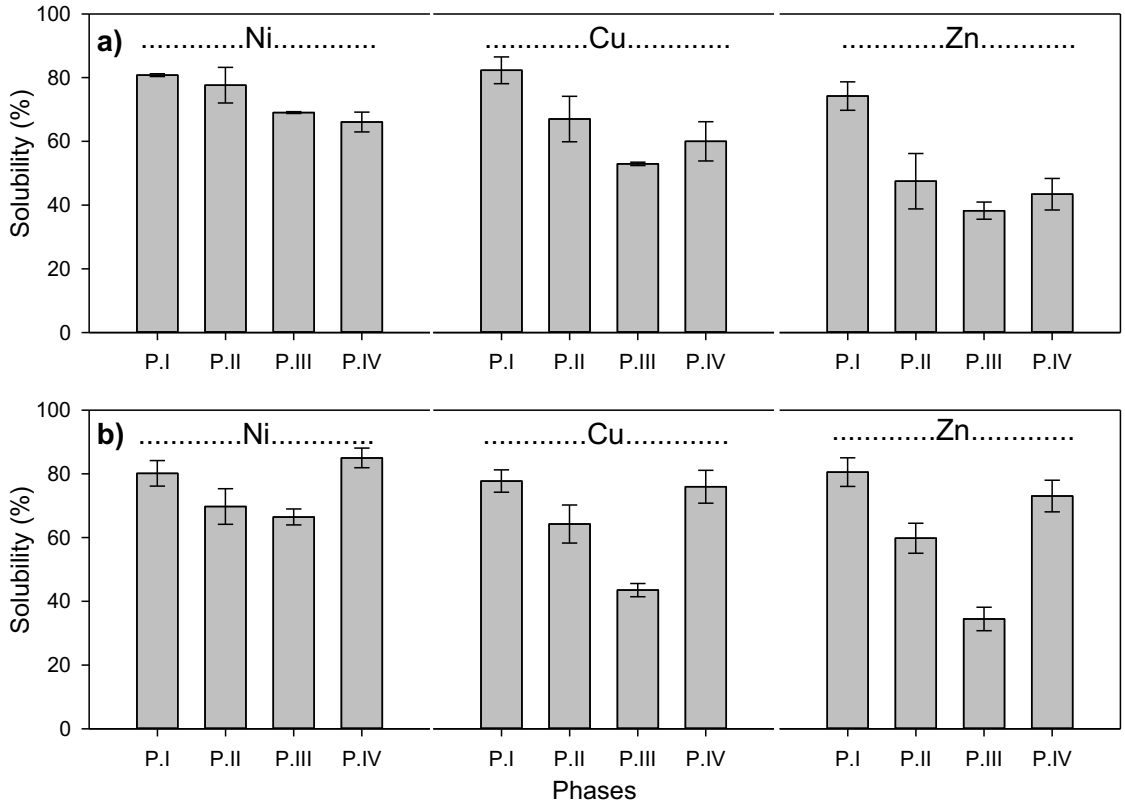

Figure 3. 13. Influence of air-assisted ultrasonication in solubilization of heavy metals from digestate: Control reactor (a); Test reactor (b)

Therefore, with this mechanism, organic matters in the system could enhance the generation of more OH• radicals, that facilitate the destruction of organic compounds. As a consequence of degradation of organic compounds, metals release efficiency would increase in the aqueous phase.

3.7. Visual MINTEQ 3.1 Prediction of Dissolved, Adsorbed, and Precipitated Species of Potentially Toxic Metals

The metal distribution profiles in the dissolved, adsorbed and precipitated species were modeled using Visual MINTEQ 3.1, in pH range of 5.5 to 7 to assess the restricting parameters for the

solubility of HMs during the digestion process (Figure 3.10). Regarding Cr, when the pH increased from 5.5 to 6.6 no changes in the Cr speciation was observed and the maximum solubility of Cr was predicted. When the pH exceeded 6.7, decline in the dissolved portion of the Cr co-occurred with considerable generation of the precipitated form. By contrast, in the experimental results, at low pH values, considerable portion of Cr was determined in the organic bound and residual fraction of the effluent digestate. Due to lack of input information in Visual MINTEQ, complexation of Cr with recalcitrant organic matter was not considered and hence at pH values < 6.7, overestimation of Cr in dissolved form was predicted. Toward the end of the study and when the pH increased to neutral conditions, solubility of Cr in the predicted model was in accordance with the experimental results (Figures 3.5 and 3.10). In the case of Cu, major portion of the total concentration was predicted to be in the adsorbed species. When the pH increased from 5.5 to 6.6, minor portion of the Cu was predicted to be in the dissolved form. As the pH increased to more than 6.6, the dissolved from of the Cu was predicted as negligible. Experimental data verified Visual MINTEQ prediction and minor portion of the Cu was determined in the soluble fraction during the whole digestion process and major portion of the Cu was measured in the organically bound and residual fractions (Figures 3.5, 3.6 and 3.10). Predicted soluble portion of Ni and Zn revealed similar trend in response to varied pH. There was a minor decrease in soluble portion of Ni and Zn when the pH increased from 5.5 to 5.8. Major decrease in the dissolved portion of Ni and Zn were observed as the pH increased from 5.8 to 6.2. In the case of Ni, the predicted results were consistent with the experimental findings. The increased dissolved concentration of Ni at low pH, in both predicted outcomes by Visual MINTEQ and experimental results, can be linked to the WAS degradation, and hence, desorption of Ni co-occurred with protonation of the negatively charged functional groups in the surface of the particulate organic matter. In the case of Zn, deviation in the dissolved fraction of the predicted and experimental results can be described by deficiency of the input parameters to the software. Therefore, at low pH, high concentration of Zn was predicted in the dissolved from, while, experimental results revealed that solubility of Zn was lower than 9% during the whole digestion process. Similar to the experimental results, inclusion of the VFAs in the model revealed that VFAs had limited influence on the speciation of HMs. On the other hand, adsorption is the process that might decrease metal concentrations in the dissolved form. WAS is one of the excellent sorbents for HMs due to its large surface area, which is covered with negatively charged functional groups.

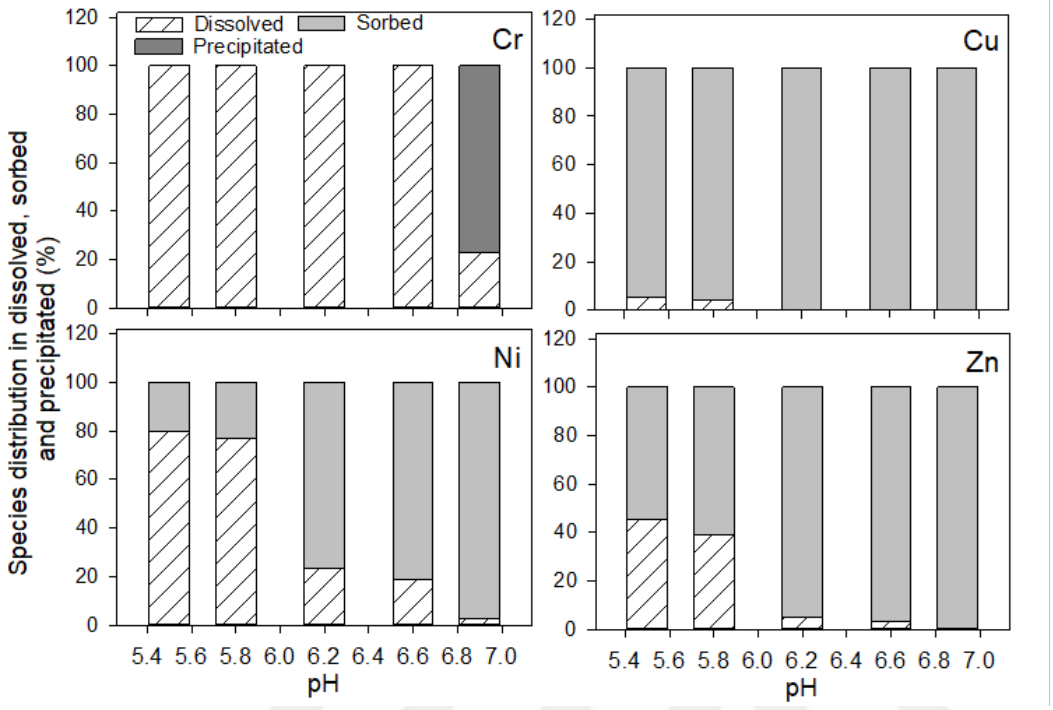

Figure 3. 14. Visual MINTEQ prediction of potentially toxic metals in dissolved, adsorbed and precipitated species

Therefore, pH and adsorption are the most contributing parameters influencing the distribution of Cu, Ni and Zn in the dissolved and adsorbed species. Simulation of the Cr showed different pattern displayed by other metals (Cu, Ni and Zn) and when the pH increased to neutral, most of the Cr species presented in the precipitated form rather than adsorbed species.

3.8. Future perspective

In recent years, wastewater treatment is increasingly becoming a challenge due to involvement of various pollutants such as HMs. Considerable portion of HMs remain in sewage sludge in common wastewater treatment processes and released to the environment along with the treated effluents. Release of HMs into the environment will pose negative impact on human health, terrestrial and aquatic life, and hence their presence in the environment is of great concern. The in-depth study of HMs bioleaching is a matter of great discussion not only in terms of comprehension of biological HM removal processes but also

upgrading the efficiency of bioleaching processes through optimization of operational parameters. In this regard, the future platform in HM bioleaching and key approaches for sustainability of the processes should be investigated.

Anaerobic bioleaching processes require long time and human capital for set up and operation and key approaches to guarantee the sustainability of this methodology. Therefore, research in lab-scale should be continued to made provision regarding the anaerobic bioleaching processes. Up-scaling this methodology, needs to be started with a strategy funding for instance from a public sector entity for providing the equipment. Moreover, this needs to be matched to more sustained funding source because cost of the running of anaerobic bioleaching processes might be high. In this study, the required cost for the anaerobic bioleaching process exceeded 71% of the total cost required for the production of stabilized digestate with low metal content. Moreover, specialized staff for the operation of the reactors, expensive equipment installation and equipment maintenance in a long-run, will further increase the implementation cost of the anaerobic bioleaching processes.

Although anaerobic bioleaching can be considered as a promising method for de-metallization of WAS, without using chemical agents, large number of EPS embedded in WAS will make this method less efficient. It is well-known that EPS in wide variety of microbial communities, increases with an increase in retention time, and hence more EPS is generated under the endogenous decay conditions (Sesay et al., 2006). In conventional WWTPs, high consumption of organic carbon presented in the wastewater and generation of EPS due to long SRTs, e.g., 10-20d, decrease the energy potential and sludge biodegradability (Ge et al., 2017).

The drive towards sustainability might be achieved by development of integrated processes that valorize the whole sewage sludge hence strengthen the idea of zero (or limited) water waste. In this regard, a process of combining AD and air-assisted ultrasonication as a post-treatment tool, introduces a novel method for HM removal and evaluates it against the conventional HM removal from sewage sludge. Another key approach is to valorize the wastewater, is to redirect carbon and nutrients to the WAS streams, while keeping the current treatment quality. In subsequent processes high concentration of biodegradable organic carbon can be converted to VFAs which are organic ligands for HM extraction. This might

be achieved with using sludge from WWTP in which biological processes accomplished via adsorption-biodegradation processes, termed high rate activated sludge (Cagnetta et al., 2016). This process is operated with high loading rates of 2-10 kg BOD/kgVS·d, low hydraulic retention time (HRT) of 15-30 min and SRT between several hours up to 1 day. Proportion to decrease the SRT from several days to a few hours, low concentration of EPS will be generated in the sludge. Moreover, the sludge would contain high biodegradable organic matter that can be easily digested to VFAs, which would perhaps enhance the HM bioleaching efficiency during the anaerobic digestion process and offset the total energy consumption for HMs removal.

4. CONCLUSION

The aim of this thesis study was to (1) produce stabilized sewage sludge with low metal content (2) recover the energy content of sewage sludge in the form of CH_4, by operating the fermentation based anaerobic bioleaching process. In this study, the obtained results, revealed that during the AD, the ligand effects of VFAs, in the leachability of metals, other than Ni, e.g., Cr, Cu, and Zn in WAS, was negligible. Low solubilization of HMs in AD process was correlated to the partitioning of HMs in the organic bound and residual fractions of sewage sludge and digestate. This was confirmed by sequential extraction scheme for metals and revealed that the highest concentrations of target metals, e.g., Cr, Cu, and Zn were existed in organically bound and residual fractions. Compared to aforementioned metals, deviation in the fractionation of Ni was observed and it was mostly distributed between organically bound, residual, and exchangeable fractions. Although AD process slightly enhanced the solubility of target metals, it was concluded that anaerobic bioleaching processes, by its own is not enough for de-metallization of sewage sludge. In this respect, hybrid technology including biological, chemical and physical processes are required to produce stabilized sewage sludge with low metal content. Therefore, as a post-treatment process, the abiotic test performed by using Fenton's reagent (H_2O_2 + Fe^{+2}), and it was revealed that for de-metallization of WAS, pH should be decreased to 2. In this regard, at acidic conditions (pH:2), application of the post-treatment processes using Fenton's reagent, could increase the solubility of HMs and 74% of Ni, 88% of Cu, 57% of Zn and 12% of Cr were leached into the liquid fraction. However, Fenton reaction at neutral pH did not increase the leachability of HMs due to precipitation of Fe^{+2} and target HMs. In this study and in the 2nd post-treatment processes, application of the air-assisted ultrasonication, considerably increased the solubilization of HMs and more than 83% of Ni, 82% of Cu and 80% of Zn were leached into the liquid fraction of the digestate. The results of this study, recommend that further studies with large-scale reactors are needed to demonstrate the energy efficiency of the process more distinctly.

5. REFERENCES

Abusoglu, A., Demir, S., Kanoglu, M., 2013. Causes, Impacts and Solutions to Global Warming. Causes, Impacts Solut. to Glob. Warm. https://doi.org/10.1007/978-1-4614-7588-0

Ahring, B.K., Ibrahim, A.A., Mladenovska, Z., 2001. Effect of temperature increase from 55 to 65°C on performance and microbial population dynamics of an anaerobic reactor treating cattle manure. Water Res. 35, 2446–2452. https://doi.org/10.1016/S0043-1354(00)00526-1

Alvarez, E.A., Mochon, M.C., Sanchez, J.C.J., Rodriguez, M.T., 2002. Heavy metal extractable forms in sludge from wastewater treatment plants. Chemosphere 47, 765–775. https://doi.org/10.1016/S0045-6535(02)00021-8

Amani, T., Nosrati, M., Sreekrishnan, T.R., 2010. Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects — a review. Environ. Rev. 18, 255–278. https://doi.org/10.1139/A10-011

Anjum, F., Shahid, M., Akcil, A., 2012. Hydrometallurgy Biohydrometallurgy techniques of low grade ores : A review on black shale. Hydrometallurgy 117–118, 1–12. https://doi.org/10.1016/j.hydromet.2012.01.007

Antoniadis, V., Levizou, E., Shaheen, S.M., Ok, Y.S., Sebastian, A., Baum, C., Prasad, M.N.V., Wenzel, W.W., Rinklebe, J., 2017. Trace elements in the soil-plant interface: Phytoavailability, translocation, and phytoremediation–A review. Earth-Science Rev. 171, 621–645. https://doi.org/10.1016/J.EARSCIREV.2017.06.005

APHA-AWWA-WEF, 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association (APHA). Washington, DC, USA.

Appels, L., Baeyens, J., Degrève, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy Combust. Sci. 34, 755–781. https://doi.org/10.1016/J.PECS.2008.06.002

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., Lens, P.N.L., 2014. Pretreatment methods to enhance anaerobic digestion of organic solid waste. Appl. Energy 123, 143–156. https://doi.org/10.1016/j.apenergy.2014.02.035

Avery, S. V, Tobin, J.M., 1993. Mechanism of adsorption of hard and soft metal ions to

Saccharomyces cerevisiae and influence of hard and soft anions. Appl. Environ. Microbiol. 59, 2851 LP – 2856.

Bárcenas-Moreno, G., Brandón, M.G., Rousk, J., Bååth, E., 2009. Adaptation of soil microbial communities to temperature: Comparison of fungi and bacteria in a laboratory experiment. Glob. Chang. Biol. 15, 2950–2957. https://doi.org/10.1111/j.1365-2486.2009.01882.x

Bayat, B., Sari, B., 2010. Comparative evaluation of microbial and chemical leaching processes for heavy metal removal from dewatered metal plating sludge. J. Hazard. Mater. 174, 763–769. https://doi.org/10.1016/J.JHAZMAT.2009.09.117

Bayrakdar, A., Molaey, R., Sürmeli, R.Ö., Sahinkaya, E., Çalli, B., 2017. Biogas production from chicken manure: Co-digestion with spent poppy straw. Int. Biodeterior. Biodegrad. 119, 205–210. https://doi.org/10.1016/j.ibiod.2016.10.058

Bezzina, J.P., Ruder, L.R., Dawson, R., Ogden, M.D., 2019. Ion exchange removal of Cu(II), Fe(II), Pb(II)and Zn(II)from acid extracted sewage sludge – Resin screening in weak acid media. Water Res. 158, 257–267. https://doi.org/10.1016/j.watres.2019.04.042

Bibby, K., Peccia, J., 2013. Identification of viral pathogen diversity in sewage sludge by metagenome analysis. Environ. Sci. Technol. 47, 1945–1951. https://doi.org/10.1021/es305181x

Blais, J.-F., Auclair, J.C., Tyagi, R.D., 1992. Cooperation between two Thiobacillus strains for heavy-metal removal from municipal sludge. Can. J. Microbiol. 38, 181–187. https://doi.org/10.1139/m92-031

Borggaard, O.K., Holm, P.E., Strobel, B.W., 2019. Potential of dissolved organic matter (DOM) to extract As, Cd, Co, Cr, Cu, Ni, Pb and Zn from polluted soils: A review. Geoderma 343, 235–246. https://doi.org/10.1016/J.GEODERMA.2019.02.041

Bratina, B., Šorgo, A., Kramberger, J., Ajdnik, U., Zemljič, L.F., Ekart, J., Šafarič, R., 2016. From municipal/industrial wastewater sludge and FOG to fertilizer: A proposal for economic sustainable sludge management. J. Environ. Manage. 183, 1009–1025. https://doi.org/10.1016/j.jenvman.2016.09.063

Brown, M.J., Lester, J.N., 1982. Role of bacterial extracellular polymers in metal uptake in pure bacterial culture and activated sludge—II Effects of mean cell retention time. Water Res.

16, 1549–1560. https://doi.org/10.1016/0043-1354(82)90207-X

Cagnetta, C., Coma, M., Vlaeminck, S.E., Rabaey, K., 2016. Production of carboxylates from high rate activated sludge through fermentation. Bioresour. Technol. 217, 165–172. https://doi.org/10.1016/j.biortech.2016.03.053

Camargo, F.P., Sérgio Tonello, P., dos Santos, A.C.A., Duarte, I.C.S., 2016. Removal of toxic metals from sewage sludge through chemical, physical, and biological treatments: A review. Water, Air, Soil Pollut. 227, 433. https://doi.org/10.1007/s11270-016-3141-3

Carrère, H., Dumas, C., Battimelli, A., Batstone, D.J., Delgenès, J.P., Steyer, J.P., Ferrer, I., 2010. Pretreatment methods to improve sludge anaerobic degradability: A review. J. Hazard. Mater. 183, 1–15. https://doi.org/10.1016/j.jhazmat.2010.06.129

Chari, B.P., Halden, R.U., 2012. Validation of mega composite sampling and nationwide mass inventories for 26 previously unmonitored contaminants in archived biosolids from the U.S National Biosolids Repository. Water Res. 46, 4814–4824. https://doi.org/10.1016/j.watres.2012.06.017

Chen, S.-Y., Pan, S.-H., 2010. Simultaneous metal leaching and sludge digestion by thermophilic microorganisms: Effect of solids content. J. Hazard. Mater. 179, 340–347. https://doi.org/10.1016/J.JHAZMAT.2010.03.011

Chu, C.P., Chang, B.-V., Liao, G.S., Jean, D.S., Lee, D.J., 2001. Observations on changes in ultrasonically treated waste-activated sludge. Water Res. 35, 1038–1046. https://doi.org/https://doi.org/10.1016/S0043-1354(00)00338-9

Clark, M.E., Batty, J.D., van Buuren, C.B., Dew, D.W., Eamon, M.A., 2006. Biotechnology in minerals processing: Technological breakthroughs creating value. Hydrometallurgy 83, 3–9. https://doi.org/https://doi.org/10.1016/j.hydromet.2006.03.046

Comte, S., Guibaud, G., Baudu, M., 2006. Biosorption properties of extracellular polymeric substances (EPS) resulting from activated sludge according to their type: Soluble or bound. Process Biochem. 41, 815–823. https://doi.org/10.1016/j.procbio.2005.10.014

Constable, P.D., Hinchcliff, K.W., Done, S.H., Grünberg, W.B.T.-V.M. (Eleventh E. (Eds.), 2017. 8 - Diseases of the Alimentary Tract–Ruminant. W.B. Saunders, pp. 436–621. https://doi.org/https://doi.org/10.1016/B978-0-7020-5246-0.00008-5

Costley, S.., Wallis, F.., 2001. Bioremediation of heavy metals in a synthetic wastewater using a rotating biological contactor. Water Res. 35, 3715–3723. https://doi.org/10.1016/S0043-1354(01)00072-0

Couillard, D., Mercier, G., 1990. Bacterial leaching of heavy metals from sewage sludge—Bioreactors comparison. Environ. Pollut. 66, 237–252. https://doi.org/10.1016/0269-7491(90)90004-V

Dąbrowska, L., 2012. Speciation of heavy metals in sewage sludge after mesophilic and thermophilic anaerobic digestion. Chem. Pap. https://doi.org/10.2478/s11696-011-0128-9

Dąbrowska, L., Rosińska, A., 2012. Change of PCBs and forms of heavy metals in sewage sludge during thermophilic anaerobic digestion. Chemosphere 88, 168–173. https://doi.org/10.1016/J.CHEMOSPHERE.2012.02.073

Dai, X., Xu, Y., Dong, B., 2017. Effect of the micron-sized silica particles (MSSP) on biogas conversion of sewage sludge. Water Res. 115, 220–228. https://doi.org/10.1016/j.watres.2017.02.064

Dai, Z., Heidrich, E.S., Dolfing, J., Jarvis, A.P., 2019. Determination of the Relationship between the Energy Content of Municipal Wastewater and Its Chemical Oxygen Demand. Environ. Sci. Technol. Lett. 6, 396–400. https://doi.org/10.1021/acs.estlett.9b00253

Demirbas, A., Edris, G., Alalayah, W.M., 2017. Sludge production from municipal wastewater treatment in sewage treatment plant. Energy Sources, Part A Recover. Util. Environ. Eff. 39, 999–1006. https://doi.org/10.1080/15567036.2017.1283551

Demirel, B., Yenigün, O., 2002. Two-phase anaerobic digestion processes: A review. J. Chem. Technol. Biotechnol. 77, 743–755. https://doi.org/10.1002/jctb.630

Dong, B., Liu, X., Dai, L., Dai, X., 2013. Changes of heavy metal speciation during high-solid anaerobic digestion of sewage sludge. Bioresour. Technol. 131, 152–158. https://doi.org/10.1016/j.biortech.2012.12.112

Fang, L., Wei, X., Cai, P., Huang, Q., Chen, H., Liang, W., Rong, X., 2011. Role of extracellular polymeric substances in Cu(II) adsorption on Bacillus subtilis and Pseudomonas putida. Bioresour. Technol. 102, 1137–1141. https://doi.org/https://doi.org/10.1016/j.biortech.2010.09.006

Fang, W., Wei, Y., Liu, J., Kosson, D.S., van der Sloot, H.A., Zhang, P., 2016. Effects of aerobic and anaerobic biological processes on leaching of heavy metals from soil amended with sewage sludge compost. Waste Manag. 58, 324–334. https://doi.org/10.1016/j.wasman.2016.07.036

Feng, X., Lei, H., Deng, J., Yu, Q., Li, H., 2009. Physical and chemical characteristics of waste activated sludge treated ultrasonically. Chem. Eng. Process. Process Intensif. 48, 187–194. https://doi.org/https://doi.org/10.1016/j.cep.2008.03.012

Fernandez, E., Alonso Peralta, F., Sanglier Contreras, G., Gonzalez-Lezcano, R., 2020. The Water Cycle in the Smart Cities Environment. https://doi.org/10.4018/978-1-7998-3817-3.ch006

Florian, D., Barnes, R.M., Knapp, G., 1998. Comparison of microwave-assisted acid leaching techniques for the determination of heavy metals in sediments, soils, and sludges. Fresenius. J. Anal. Chem. 362, 558–565. https://doi.org/10.1007/s002160051124

Foladori, P., Tamburini, S., Bruni, L., 2010. Bacteria permeabilisation and disruption caused by sludge reduction technologies evaluated by flow cytometry. Water Res. 44, 4888–4899. https://doi.org/https://doi.org/10.1016/j.watres.2010.07.030

Formentini, T.A., Legros, S., Fernandes, C.V.S., Pinheiro, A., Le Bars, M., Levard, C., Mallmann, F.J.K., da Veiga, M., Doelsch, E., 2017. Radical change of Zn speciation in pig slurry amended soil: Key role of nano-sized sulfide particles. Environ. Pollut. 222, 495.

Fuentes, A., Lloréns, M., Sáez, J., Isabel Aguilar, M., Ortuño, J.F., Meseguer, V.F., 2008. Comparative study of six different sludges by sequential speciation of heavy metals. Bioresour. Technol. 99, 517–525. https://doi.org/10.1016/J.BIORTECH.2007.01.025

Fullana, A., Conesa, J.A., Font, R., Sidhu, S., 2004. Formation and destruction of chlorinated pollutants during sewage sludge incineration. Environ. Sci. Technol. 38, 2953–2958. https://doi.org/10.1021/es034896u

Gadd, G.M., 2009. Biosorption: Critical review of scientific rationale, environmental importance and significance for pollution treatment. J. Chem. Technol. Biotechnol. 84, 13–28. https://doi.org/10.1002/jctb.1999

Gadd, G.M., 2008. Accumulation and Transformation of Metals by Microorganisms. Biotechnol.

Second. Complet. Revis. Ed. 10–12, 225–264. https://doi.org/10.1002/9783527620999.ch9k

Gagliano, M.C., Braguglia, C.M., Gianico, A., Mininni, G., Nakamura, K., Rossetti, S., 2015. Thermophilic anaerobic digestion of thermal pretreated sludge: Role of microbial community structure and correlation with process performances. Water Res. 68, 498–509. https://doi.org/10.1016/j.watres.2014.10.031

Garrido-Baserba, M., Molinos-Senante, M., Abelleira-Pereira, J.M., Fdez-Güelfo, L.A., Poch, M., Hernández-Sancho, F., 2015. Selecting sewage sludge treatment alternatives in modern wastewater treatment plants using environmental decision support systems. J. Clean. Prod. 107, 410–419. https://doi.org/10.1016/J.JCLEPRO.2014.11.021

Ge, H., Batstone, D.J., Mouiche, M., Hu, S., Keller, J., 2017. Nutrient removal and energy recovery from high-rate activated sludge processes – Impact of sludge age. Bioresour. Technol. 245, 1155–1161. https://doi.org/10.1016/j.biortech.2017.08.115

Golab, Z., Breitenbach, M., Jezierski, A., 1995. Sites of copper binding in Streptomyces pilosus. Water. Air. Soil Pollut. 82, 713–721. https://doi.org/10.1007/BF00479421

Gustafsson, J.P., Persson, I., Oromieh, A.G., Van Schaik, J.W.J., Sjöstedt, C., Kleja, D.B., 2014. Chromium(III) complexation to natural organic matter: Mechanisms and modeling. Environ. Sci. Technol. 48, 1753–1761. https://doi.org/10.1021/es404557e

Ha, J., Gélabert, A., Spormann, A.M., Brown, G.E., 2010. Role of extracellular polymeric substances in metal ion complexation on Shewanella oneidensis: Batch uptake, thermodynamic modeling, ATR-FTIR, and EXAFS study. Geochim. Cosmochim. Acta 74, 1–15. https://doi.org/10.1016/j.gca.2009.06.031

Harrison, J.J., Ceri, H., Turner, R.J., 2007. Multimetal resistance and tolerance in microbial biofilms. Nat. Rev. Microbiol. 5, 928–938. https://doi.org/10.1038/nrmicro1774

Hei, L., Jin, P., Zhu, X., Ye, W., Yang, Y., 2016. Characteristics of speciation of heavy metals in municipal sewage sludge of Guangzhou as fertilizer. Procedia Environ. Sci. 31, 232–240. https://doi.org/10.1016/j.proenv.2016.02.031

Hidaka, T., Arai, S., Okamoto, S., Uchida, T., 2013. Anaerobic co-digestion of sewage sludge with shredded grass from public green spaces. Bioresour. Technol. 130, 667–672. https://doi.org/10.1016/J.BIORTECH.2012.12.068

Houtmeyers, S., Degrève, J., Willems, K., Dewil, R., Appels, L., 2014. Comparing the influence of low power ultrasonic and microwave pre-treatments on the solubilisation and semi-continuous anaerobic digestion of waste activated sludge. Bioresour. Technol. 171, 44–49. https://doi.org/10.1016/J.BIORTECH.2014.08.029

Huang, H., Yuan, X., 2016. The migration and transformation behaviors of heavy metals during the hydrothermal treatment of sewage sludge. Bioresour. Technol. 200, 991–998. https://doi.org/10.1016/j.biortech.2015.10.099

Hullebusch, E.D.V., Utomo, S., Zandvoort, M.H., Piet, P.N., 2005. Comparison of three sequential extraction procedures to describe metal fractionation in anaerobic granular sludges. Talanta 65, 549–558. https://doi.org/10.1016/j.talanta.2004.07.024

Inglezakis, V.J., Zorpas, A.A., Karagiannidis, A., Samaras, P., Voukkali, I., Sklari, S., 2014. European Union legislation on sewage sludge management. Fresenius Environ. Bull. 23, 635–639.

Jenkins, R.L., Scheybeler, B.J., Smith, M.L., Baird, R., Lo, M.P., Haug, R.T., Lo, M.P., Haug, R.T., Jenkins, R.L., Scheybeler, B.J., Smith, M.L., 1981. Metals removal and recovery from municipal sludge 53, 25–32.

Jung, I.S., Lovitt, R.W., 2011. Leaching techniques to remove metals and potentially hazardous nutrients from trout farm sludge. Water Res. 45, 5977–5986. https://doi.org/10.1016/J.WATRES.2011.08.062

Kang, S.-Y., Lee, J.-U., Kim, K.-W., 2007. Biosorption of Cr(III) and Cr(VI) onto the cell surface of Pseudomonas aeruginosa. Biochem. Eng. J. 36, 54–58. https://doi.org/10.1016/J.BEJ.2006.06.005

Karna, R.R., Luxton, T., Bronstein, K.E., Hoponick Redmon, J., Scheckel, K.G., 2017. State of the science review: Potential for beneficial use of waste by-products for in situ remediation of metal-contaminated soil and sediment. Crit. Rev. Environ. Sci. Technol. 47, 65–129. https://doi.org/10.1080/10643389.2016.1275417

Kim, D.-H., Jeong, E., Oh, S.-E., Shin, H.-S., 2010. Combined (alkaline + ultrasonic) pretreatment effect on sewage sludge disintegration. Water Res. 44, 3093–3100. https://doi.org/10.1016/J.WATRES.2010.02.032

Latif, M.A., Mehta, C.M., Batstone, D.J., 2017. Influence of low pH on continuous anaerobic digestion of waste activated sludge. Water Res. 113, 42–49. https://doi.org/10.1016/J.WATRES.2017.02.002

Ledin, M., 2000. Accumulation of metals by microorganisms — processes and importance for soil systems. Earth-Science Rev. 51, 1–31. https://doi.org/10.1016/S0012-8252(00)00008-8

Legros, S., Guiresse, M., Doelsch, E., 2017. Anaerobic digestion alters copper and zinc speciation. Environ. Sci. Technol. 51, 10326–10334. https://doi.org/10.1021/acs.est.7b01662

Leng, L., Yuan, X., Shao, J., Huang, H., Wang, H., Li, H., Chen, X., Zeng, G., 2016. Study on demetalization of sewage sludge by sequential extraction before liquefaction for the production of cleaner bio-oil and bio-char. Bioresour. Technol. 200, 320–327. https://doi.org/10.1016/j.biortech.2015.10.040

Li, D., Zhou, Y., Tan, Y., Pathak, S., Majid, M.B.A., Ng, W.J., 2016. Alkali-solubilized organic matter from sludge and its degradability in the anaerobic process. Bioresour. Technol. 200, 579–586. https://doi.org/10.1016/j.biortech.2015.10.083

Li, J., Zhang, M., Ye, Z., Yang, C., 2019. Effect of manganese oxide-modified biochar addition on methane production and heavy metal speciation during the anaerobic digestion of sewage sludge. J. Environ. Sci. 76, 267–277. https://doi.org/10.1016/J.JES.2018.05.009

Li, W.-W., Yu, H.-Q., 2014. Insight into the roles of microbial extracellular polymer substances in metal biosorption. Bioresour. Technol. 160, 15–23. https://doi.org/10.1016/J.BIORTECH.2013.11.074

Li, Y., Yuan, X., Wu, Z., Wang, H., Xiao, Z., Wu, Y., Chen, X., Zeng, G., 2016. Enhancing the sludge dewaterability by electrolysis/electrocoagulation combined with zero-valent iron activated persulfate process. Chem. Eng. J. 303, 636–645. https://doi.org/10.1016/J.CEJ.2016.06.041

Lin, H., Wang, C., Zhao, H., Chen, G., Chen, X., 2020. A subcellular level study of copper speciation reveals the synergistic mechanism of microbial cells and EPS involved in copper binding in bacterial biofilms. Environ. Pollut. 263, 114485. https://doi.org/10.1016/j.envpol.2020.114485

Liu, X., Wang, W., Gao, X., Zhou, Y., Shen, R., 2012. Effect of thermal pretreatment on the physical and chemical properties of municipal biomass waste. Waste Manag. 32, 249–255. https://doi.org/10.1016/j.wasman.2011.09.027

Logan, T.J., Harrison, B.J., 1995. Physical Characteristics of Alkaline Stabilized Sewage Sludge (N-Viro Soil) and Their Effects on Soil Physical Properties. J. Environ. Qual. 24, 153–164. https://doi.org/10.2134/jeq1995.00472425002400010022x

López, A., Lázaro, N., Priego, J.M., Marqués, A.M., 2000. Effect of pH on the biosorption of nickel and other heavy metals by Pseudomonas fluorescens 4F39. J. Ind. Microbiol. Biotechnol. 24, 146–151. https://doi.org/10.1038/sj.jim.2900793

Ma, S. jia, Ma, H. jun, Hu, H. dong, Ren, H. qiang, 2019. Effect of mixing intensity on hydrolysis and acidification of sewage sludge in two-stage anaerobic digestion: Characteristics of dissolved organic matter and the key microorganisms. Water Res. 148, 359–367. https://doi.org/10.1016/j.watres.2018.10.058

Malik, O.A., Hsu, A., Johnson, L.A., de Sherbinin, A., 2015. A global indicator of wastewater treatment to inform the Sustainable Development Goals (SDGs). Environ. Sci. Policy 48, 172–185. https://doi.org/10.1016/j.envsci.2015.01.005

Marcato, C.-E., Pinelli, E., Cecchi, M., Winterton, P., Guiresse, M., 2009. Bioavailability of Cu and Zn in raw and anaerobically digested pig slurry. Ecotoxicol. Environ. Saf. 72, 1538–1544. https://doi.org/10.1016/J.ECOENV.2008.12.010

Mark Gerard Healy, Racjel M Clarke, Dara Peyton, 2012. Resource recovery from sewage sludge.

Meulepas, R.J.W., Gonzalez-gil, G., Melese, F., Witharana, A., Saikaly, P.E., Lens, P.N.L., 2015. Anaerobic bioleaching of metals from waste activated sludge. Sci. Total Environ. 514, 60–67. https://doi.org/10.1016/j.scitotenv.2014.12.073

Molaey, R., Bayrakdar, A., Sürmeli, R.Ö., Çalli, B., 2018. Anaerobic digestion of chicken manure: Mitigating process inhibition at high ammonia concentrations by selenium supplementation. Biomass and Bioenergy 108, 439–446. https://doi.org/10.1016/J.BIOMBIOE.2017.10.050

Molaey, R., Yesil, H., Calli, B., Tugtas, A.E., 2021a. Influence of volatile fatty acids in

anaerobic bioleaching of potentially toxic metals. J. Environ. Manage. 285, 112118. https://doi.org/10.1016/j.jenvman.2021.112118

Molaey, R., Yesil, H., Calli, B., Tugtas, A.E., 2021b. Enhanced heavy metal leaching from sewage sludge through anaerobic fermentation and air-assisted ultrasonication. Chemosphere 279, 130548. https://doi.org/https://doi.org/10.1016/j.chemosphere.2021.130548

Möller, K., 2015. Effects of anaerobic digestion on soil carbon and nitrogen turnover, N emissions, and soil biological activity. A review. Agron. Sustain. Dev. 35, 1021–1041. https://doi.org/10.1007/s13593-015-0284-3

Mullen, M.D., Wolf, D.C., Ferris, F.G., Beveridge, T.J., Flemming, C.A., Bailey, G.W., 1989. Bacterial sorption of heavy metals. Appl. Environ. Microbiol. 55, 3143 LP – 3149.

Neyens, E., Baeyens, J., 2003. A review of thermal sludge pre-treatment processes to improve dewaterability. J. Hazard. Mater. 98, 51–67. https://doi.org/10.1016/S0304-3894(02)00320-5

Panda, S., Akcil, A., Pradhan, N., Deveci, H., 2015. Current scenario of chalcopyrite bioleaching: A review on the recent advances to its heap-leach technology. Bioresour. Technol. 196, 694–706. https://doi.org/10.1016/j.biortech.2015.08.064

Panina, N.S., Belyaev, A.N., Simanova, S.A., 2002. Carboxylic acids and their anions. Acid and ligand properties. Russ. J. Gen. Chem. 72, 91–94. https://doi.org/10.1023/A:1015353530785

Papirio, S., Frunzo, L., Mattei, M.R., Ferraro, A., Race, M., D'Acunto, B., Pirozzi, F., Esposito, G., 2017. Heavy metal removal from wastewaters by biosorption: Mechanisms and modeling, in: Rene, E.R., Sahinkaya, E., Lewis, A., Lens, P.N.L. (Eds.), Sustainable Heavy Metal Remediation: Volume 1: Principles and Processes. Springer International Publishing, Cham, pp. 25–63. https://doi.org/10.1007/978-3-319-58622-9_2

Pathak, A., Dastidar, M.G., Sreekrishnan, T.R., 2009. Bioleaching of heavy metals from sewage sludge: A review. J. Environ. Manage. 90, 2343–2353. https://doi.org/10.1016/j.jenvman.2008.11.005

Pathak, A., Morrison, L., Healy, M.G., 2017. Catalytic potential of selected metal ions for

bioleaching, and potential techno-economic and environmental issues: A critical review. Bioresour. Technol. 229, 211–221. https://doi.org/10.1016/J.BIORTECH.2017.01.001

Peccia, J., Westerhoff, P., 2015. We Should Expect More out of Our Sewage Sludge. Environ. Sci. Technol. 49, 8271–8276. https://doi.org/10.1021/acs.est.5b01931

Pei, D., Xiao, C., Hu, Q., Tang, J., 2016. Electrokinetic Gathering and Removal of Heavy Metals from Sewage Sludge by Ethylenediamine Chelation. Procedia Environ. Sci. 31, 725–734. https://doi.org/10.1016/J.PROENV.2016.02.058

Peng, G., Tian, G., Liu, J., Bao, Q., Zang, L., 2011. Removal of heavy metals from sewage sludge with a combination of bioleaching and electrokinetic remediation technology. Desalination 271, 100–104. https://doi.org/10.1016/j.desal.2010.12.015

Pradhan, N., Nathsarma, K.C., Srinivasa Rao, K., Sukla, L.B., Mishra, B.K., 2008. Heap bioleaching of chalcopyrite: A review. Miner. Eng. 21, 355–365. https://doi.org/https://doi.org/10.1016/j.mineng.2007.10.018

Rao, P.V., Baral, S.S., 2011. Experimental design of mixture for the anaerobic co-digestion of sewage sludge. Chem. Eng. J. 172, 977–986. https://doi.org/10.1016/J.CEJ.2011.07.010

Razavi, A.S., Kakar, F., Koupaie, E.H., Elbeshbishy, E., Hafez, H., Global, G., 2021. Biomethane production improvement by hydrothermal pretreatment of thickened waste activated sludge. Water Sci. Technol. 83, 487–500. https://doi.org/10.2166/wst.2020.598

Rezza, I., Salinas, E., Elorza, M., Sanz de Tosetti, M., Donati, E., 2001. Mechanisms involved in bioleaching of an aluminosilicate by heterotrophic microorganisms. Process Biochem. 36, 495–500. https://doi.org/10.1016/S0032-9592(00)00164-3

Rodriguez, C., Alaswad, A., Benyounis, K.Y., Olabi, A.G., 2017. Pretreatment techniques used in biogas production from grass. Renew. Sustain. Energy Rev. 68, 1193–1204. https://doi.org/10.1016/J.RSER.2016.02.022

Salehizadeh, H., Shojaosadati, S.A., 2003. Removal of metal ions from aqueous solution by polysaccharide produced from Bacillus firmus. Water Res. 37, 4231–4235. https://doi.org/10.1016/S0043-1354(03)00418-4

Salihoglu, N.K., Pinarli, V., Salihoglu, G., 2007. Solar drying in sludge management in Turkey. Renew. Energy 32, 1661–1675. https://doi.org/10.1016/j.renene.2006.08.001

Samolada, M.C., Zabaniotou, A.A., 2014. Comparative assessment of municipal sewage sludge incineration, gasification and pyrolysis for a sustainable sludge-to-energy management in Greece. Waste Manag. 34, 411–420. https://doi.org/10.1016/j.wasman.2013.11.003

Selling, R., Håkansson, T., Björnsson, L., 2008. Two-stage anaerobic digestion enables heavy metal removal. Water Sci. Technol. 57, 553–558. https://doi.org/10.2166/wst.2008.054

Sesay, M.L., Özcengiz, G., Dilek Sanin, F., 2006. Enzymatic extraction of activated sludge extracellular polymers and implications on bioflocculation. Water Res. 40, 1359–1366. https://doi.org/https://doi.org/10.1016/j.watres.2006.01.045

Sheng, G.P., Xu, J., Luo, H.W., Li, W.W., Li, W.H., Yu, H.Q., Xie, Z., Wei, S.Q., Hu, F.C., 2013. Thermodynamic analysis on the binding of heavy metals onto extracellular polymeric substances (EPS) of activated sludge. Water Res. 47, 607–614. https://doi.org/10.1016/j.watres.2012.10.037

Singh, R.P., Agrawal, M., 2008. Potential benefits and risks of land application of sewage sludge. Waste Manag. 28, 347–358. https://doi.org/10.1016/J.WASMAN.2006.12.010

Singh, R.P., Agrawal, M., 2007. Effects of sewage sludge amendment on heavy metal accumulation and consequent responses of Beta vulgaris plants. Chemosphere 67, 2229–2240. https://doi.org/https://doi.org/10.1016/j.chemosphere.2006.12.019

Singh, S., Cameotra, S.S., 2015. Anaerobic Bioleaching by Acidophilic Bacterial Strains BT - Environmental Microbial Biotechnology, in: Sukla, L.B., Pradhan, N., Panda, S., Mishra, B.K. (Eds.), . Springer International Publishing, Cham, pp. 179–201. https://doi.org/10.1007/978-3-319-19018-1_10

Smith, S.R., Durham, E., 2002. Nitrogen release and fertiliser value of thermally-dried biosolids. Water Environ. J. 16, 121–126. https://doi.org/10.1111/j.1747-6593.2002.tb00382.x

Soleimani, M., Hajabbasi, M.A., Afyuni, M., Akbar, S., Jensen, J.K., Holm, P.E., Borggaard, O.K., 2010. Comparison of Natural Humic Substances and Synthetic Ethylenediaminetetraacetic Acid and Nitrilotriacetic Acid as Washing Agents of a Heavy Metal-Polluted Soil. J. Environ. Qual. 39, 855–862. https://doi.org/10.2134/jeq2009.0292

Sommers, L.E., 1977. Chemical Composition of Sewage Sludges and Analysis of Their Potential Use as Fertilizers. J. Environ. Qual. 6, 225–232.

https://doi.org/10.2134/jeq1977.00472425000600020026x

Sponza, D.T., 2002. Extracellular polymer substances and physicochemical properties of flocs in steady and unsteady-state activated sludge systems. Process Biochem. 37, 983–998. https://doi.org/10.1016/S0032-9592(01)00306-5

Srinath, T., Verma, T., Ramteke, P.W., Garg, S.K., 2002. Chromium (VI) biosorption and bioaccumulation by chromate resistant bacteria. Chemosphere 48, 427–435. https://doi.org/10.1016/S0045-6535(02)00089-9

Strobel, B.W., 2001. Influence of vegetation on low-molecular-weight carboxylic acids in soil solution—a review. Geoderma 99, 169–198. https://doi.org/10.1016/S0016-7061(00)00102-6

Stroot, P.G., McMahon, K.D., Mackie, R.I., Raskin, L., 2001. Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions—I. digester performance. Water Res. 35, 1804–1816. https://doi.org/10.1016/S0043-1354(00)00439-5

Suanon, F., Sun, Q., Mama, D., Li, J., Dimon, B., Yu, C.P., 2016. Effect of nanoscale zero-valent iron and magnetite (Fe3O4) on the fate of metals during anaerobic digestion of sludge. Water Res. 88, 897–903. https://doi.org/10.1016/j.watres.2015.11.014

Tang, Y., Dai, X., Dong, B., Guo, Y., Dai, L., 2020. Humification in extracellular polymeric substances (EPS) dominates methane release and EPS reconstruction during the sludge stabilization of high-solid anaerobic digestion. Water Res. 175, 115686. https://doi.org/10.1016/j.watres.2020.115686

Tessier, A., Campbell, P.G.C., Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem. 51, 844–851. https://doi.org/10.1021/ac50043a017

Tyagi, R.D., Meunier, N., Blais, J.F., 1996. Simultaneous sewage sludge digestion and metal leaching — effect of temperature. Appl. Microbiol. Biotechnol. 46, 422–431. https://doi.org/10.1007/BF00166240

Uçkun Kiran, E., Stamatelatou, K., Antonopoulou, G., Lyberatos, G., 2016. Production of biogas via anaerobic digestion. Handb. Biofuels Prod. 259–301. https://doi.org/10.1016/B978-0-08-100455-5.00010-2

Urrea, J.L., Collado, S., Oulego, P., Díaz, M., 2017. Formation and Degradation of Soluble Biopolymers during Wet Oxidation of Sludge. ACS Sustain. Chem. Eng. 5, 3011–3018. https://doi.org/10.1021/acssuschemeng.6b02664

van Hullebusch, E.D., Zandvoort, M.H., Lens, P.N.L., 2003. Metal immobilisation by biofilms: Mechanisms and analytical tools. Rev. Environ. Sci. Biotechnol. 2, 9–33. https://doi.org/10.1023/B:RESB.0000022995.48330.55

Velásquez, L., Dussan, J., 2009. Biosorption and bioaccumulation of heavy metals on dead and living biomass of Bacillus sphaericus. J. Hazard. Mater. 167, 713–716. https://doi.org/10.1016/j.jhazmat.2009.01.044

Villar, L.D., Garcia, O., 2002. Solubilization profiles of metal ions from bioleaching of sewage sludge as a function of pH. Biotechnol. Lett. 24, 611–614. https://doi.org/10.1023/A:1015010417315

Walter, I., Martínez, F., Cala, V., 2006. Heavy metal speciation and phytotoxic effects of three representative sewage sludges for agricultural uses. Environ. Pollut. 139, 507–514. https://doi.org/10.1016/j.envpol.2005.05.020

Wang, F., Lu, S., Ji, M., 2006. Components of released liquid from ultrasonic waste activated sludge disintegration. Ultrason. Sonochem. 13, 334–338. https://doi.org/https://doi.org/10.1016/j.ultsonch.2005.04.008

Wang, J., Li, Q., Li, M.-M., Chen, T.-H., Zhou, Y.-F., Yue, Z.-B., 2014. Competitive adsorption of heavy metal by extracellular polymeric substances (EPS) extracted from sulfate reducing bacteria. Bioresour. Technol. 163, 374–376. https://doi.org/10.1016/J.BIORTECH.2014.04.073

Wang, Q., Ye, L., Jiang, G., Jensen, P.D., Batstone, D.J., Yuan, Z., 2013. Free nitrous acid (FNA)-based pretreatment enhances methane production from waste activated sludge. Environ. Sci. Technol. 47, 11897–11904. https://doi.org/10.1021/es402933b

Weng, L., Temminghoff, E.J.M., Lofts, S., Tipping, E., Van Riemsdijk, W.H., 2002. Complexation with Dissolved Organic Matter and Solubility Control of Heavy Metals in a Sandy Soil. Environ. Sci. Technol. 36, 4804–4810. https://doi.org/10.1021/es0200084

Werther, J., Ogada, T., 1999. Sewage sludge combustion. Prog. Energy Combust. Sci. 25, 55–

116. https://doi.org/10.1016/S0360-1285(98)00020-3

Wilson, C.A., Novak, J.T., 2009. Hydrolysis of macromolecular components of primary and secondary wastewater sludge by thermal hydrolytic pretreatment. Water Res. 43, 4489–4498. https://doi.org/10.1016/j.watres.2009.07.022

Wong, B.-T., Show, K.Y., Lee, D.J., Lai, J.Y., 2009. Carbon balance of anaerobic granulation process: Carbon credit. Bioresour. Technol. 100, 1734–1739. https://doi.org/10.1016/J.BIORTECH.2008.09.045

Wong, P.K., Lam, K.C., So, C.M., 1993. Removal and recovery of Cu(II) from industrial effluent by immobilized cells of Pseudomonas putida II-11. Appl. Microbiol. Biotechnol. 39, 127–131. https://doi.org/10.1007/BF00166861

Wu, B., Ni, B.-J., Horvat, K., Song, L., Chai, X., Dai, X., Mahajan, D., 2017. Occurrence State and Molecular Structure Analysis of Extracellular Proteins with Implications on the Dewaterability of Waste-Activated Sludge. Environ. Sci. Technol. 51, 9235–9243. https://doi.org/10.1021/acs.est.7b02861

Wu, Y., Li, T., Yang, L., 2012. Mechanisms of removing pollutants from aqueous solutions by microorganisms and their aggregates: A review. Bioresour. Technol. 107, 10–18. https://doi.org/10.1016/j.biortech.2011.12.088

Xie, S., Ma, Y., Strong, P.J., Clarke, W.P., 2015. Fluctuation of dissolved heavy metal concentrations in the leachate from anaerobic digestion of municipal solid waste in commercial scale landfill bioreactors: The effect of pH and associated mechanisms. J. Hazard. Mater. 299, 577–583. https://doi.org/10.1016/j.jhazmat.2015.07.065

Xu, Q., Wang, H., Wang, Q., Zhang, W., Wang, D., 2019. Characterization of changes in extracellular polymeric substances and heavy metal speciation of waste activated sludge during typical oxidation solubilization processes. J. Environ. Sci. (China) 80, 146–158. https://doi.org/10.1016/j.jes.2018.12.005

Xu, Y., Lu, Y., Dai, X., Dong, B., 2017. The influence of organic-binding metals on the biogas conversion of sewage sludge. Water Res. 126, 329–341. https://doi.org/10.1016/J.WATRES.2017.09.046

Xu, Y., Lu, Y., Dai, X., Liu, M., Dai, L., Dong, B., 2018. Spatial Configuration of Extracellular

Organic Substances Responsible for the Biogas Conversion of Sewage Sludge. ACS Sustain. Chem. Eng. 6, 8308–8316. https://doi.org/10.1021/acssuschemeng.8b00313

Yaman, K., Olhan, E., 2012. A Comparative Analysis of Turkish and European Union Regulations Concerning Use of Sewage Sludge in Agriculture, in: 6th International Symposium on Advances in Science and Technology. Kuala Lumpur/Malayssia.

Yang, G., Zhang, G., Wang, H., 2015. Current state of sludge production, management, treatment and disposal in China. Water Res. 78, 60–73. https://doi.org/10.1016/j.watres.2015.04.002

Yang, W., Song, W., Li, J., Zhang, X., 2020. Bioleaching of heavy metals from wastewater sludge with the aim of land application. Chemosphere 249. https://doi.org/10.1016/j.chemosphere.2020.126134

Yesil, H., Molaey, R., Calli, B., Tugtas, A.E., 2021. Extent of bioleaching and bioavailability reduction of potentially toxic heavy metals from sewage sludge through pH-controlled fermentation. Water Res. 117303. https://doi.org/https://doi.org/10.1016/j.watres.2021.117303

Yesil, H., Tugtas, A.E., 2019. Removal of heavy metals from leaching effluents of sewage sludge via supported liquid membranes. Sci. Total Environ. 693, 133608. https://doi.org/10.1016/J.SCITOTENV.2019.133608

Yu, W., Yang, J., Shi, Yafei, Song, J., Shi, Yao, Xiao, J., Li, C., Xu, X., He, S., Liang, S., Wu, X., Hu, J., 2016. Roles of iron species and pH optimization on sewage sludge conditioning with Fenton's reagent and lime. Water Res. 95, 124–133. https://doi.org/10.1016/j.watres.2016.03.016

Zhang, M., Yang, C., Jing, Y., Li, J., 2016. Effect of energy grass on methane production and heavy metal fractionation during anaerobic digestion of sewage sludge. Waste Manag. 58, 316–323. https://doi.org/10.1016/j.wasman.2016.09.040

Zhao, H., Zhang, P., Zhang, G., Cheng, R., 2016. Enhancement of ultrasonic disintegration of sewage sludge by aeration. J. Environ. Sci. (China) 42, 163–167. https://doi.org/10.1016/j.jes.2015.08.009

Zhen, G., Lu, X., Kato, H., Zhao, Y., Li, Y.-Y., 2017. Overview of pretreatment strategies for

enhancing sewage sludge disintegration and subsequent anaerobic digestion: Current advances, full-scale application and future perspectives. Renew. Sustain. Energy Rev. 69, 559–577. https://doi.org/10.1016/J.RSER.2016.11.187

Zhen, G., Lu, X., Kobayashi, T., Li, Y.-Y., Xu, K., Zhao, Y., 2015. Mesophilic anaerobic co-digestion of waste activated sludge and Egeria densa: Performance assessment and kinetic analysis. Appl. Energy 148, 78–86. https://doi.org/10.1016/J.APENERGY.2015.03.038

Zhu, N., Qiang-Li, Guo, X., Hui-Zhang, Yu-Deng, 2014. Sequential extraction of anaerobic digestate sludge for the determination of partitioning of heavy metals. Ecotoxicol. Environ. Saf. 102, 18–24. https://doi.org/10.1016/J.ECOENV.2013.12.033