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Cam Tu Vu

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GRESE

Thèse pour obtenir le grade de
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Spécialité Eau, Sol, Environnement

Présentée et soutenue par

Cam Tu VU

Le 15 mars 2017

Comparison between granular and conventional activated sludge for trace metals elements sorption/desorption ability. Case of copper for land spreading application in France and in Vietnam.

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LIST OF ABBREVIATIONS

CEC :	Cation exchange capacity
CLSM :	Confocal scanning microscopy
COD :	Chemical oxygen demand
Con A :	ConcanavalinA conjugates - fluorescent label to polysaccharides
DOC:	Dissolved organic carbon
DS :	Dried sludge
EC :	Electronical conductivity
FA :	Fulvic acid
FITC :	Fluorescein isothiocyanate - fluorescent label to proteins
HA :	Humic acid
HM :	Heavy metal
K_d :	Distribution constant (Liner model)
K_f :	Freundlich constant of affinity
K_L :	Langmuir constant of affinity
N :	Freundlich constant of intensity
OM :	Organic matter
PEC :	Proton exchange capacity
PN :	Proteins
PS :	Polysaccharides
q_{max} :	Maximum adsorption capacity of Langmuir model
RP :	Reduction potential
TS :	Total solid
VS :	Volatile solid
WWTP :	wastewater treatment plant



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GENERAL INTRODUCTION





GENERAL INTRODUCTION

The wastewater treatment is a set of processes to convert discharged effluent with the objective to reduce the pollutants to acceptable level to give back the water into the environment without risk to health and biotopes. According to the characteristics of wastewater, the wastewater treatment plant will be established according to two main methods: chemical or physical treatment and biological treatment. Biological wastewater treatment plants use bacteria to break down and to oxidize organic matter. There are different solutions among them namely activated sludge (conventional method), biological filter, lagoons, ponds, fixed bed, and, recently, granular sludge technology. The conventional activated sludge (CAS) process is the most commonly used process for wastewater treatment in the world. The basic conventional activated sludge allows an efficient removal of carbonaceous organics and ammonium/ammonia from wastewater. However, due to the increasing concern about eutrophication, stricter regulations forced its upgrading to improve nitrogen and phosphorus removal.

In parallel, other kind of biological wastewater processes are merging, with many advantages like granular sludge processes. Granular sludge process consists in a specific biomass configuration in which biomass grows in compact aggregates (granules) without any carrier material. Granular sludge technology started to be developed about 40 years ago at Wageningen University. Compared to biomass growing in flocs, granular biomass presents several advantages that make it very attractive for wastewater treatment purposes. Granules are denser and have a stronger microbial structure than flocs. Thus, granular biomass has very high settling velocity while the typical settling velocity for flocs is at least three times lower. These excellent settling properties allow high biomass retention and lead to enhance the performance of the reactor and faster removal of the different contaminants.

Normally, wastewater contains a lot of mineral or organic contaminants from different water sources, which are susceptible to be concentrated in waste sludge. Among pollutants contained in wastewater, growing attention is being paid to the health hazards presented by the existence of heavy metals in sludge. Heavy metals are non-renewable resources and non-degradable pollutants which can accumulate on sludge during wastewater treatment. Copper pollution especially, arises for copper mining and smelting, brass manufacture, electroplating industries and excessive use of Cu based agro-chemicals. Copper along with arsenic and mercury, is recognized as the highest relative mammalian toxic and continued inhalation of copper containing spray is linked with an increase in lung cancer among exposed workers. Hence, the copper contamination in wastewater and waste sludge and copper availability in sludge recycling are very important issues concerning the risk of copper to living environment and protection of human health. In Vietnam, the rapid intensification of industrial activities including copper casting, mining chemical fertilizer production, and chemical manufacturing, handicraft production, etc., has been indicated to have introduced copper into water and soil system, especially in Delta region (Phuong et al., 2010a).

The common mechanisms for heavy metal accumulation in sludge during wastewater treatment often include adsorption, biosorption, complexation, precipitation, ion exchange, etc. According to biological wastewater treatment, both of CAS and granular sludge are highly effective and friendly materials for



adsorption to remove heavy metals ions from aqueous solutions. Researches on metals removal from water study the capability of sludge to retain, to sorb these elements, the adsorption capability could be very different and function on the physical structure of the biological sludge. So it is interesting to compare some different sludges, with very different structure, as it in the case between sludge from CAS and granular sludge. Researches on CAS and granular sludge investigate the advantages heavy metal adsorption capacity, especially on granular sludge with large surface area, dense structure, abundance and diversity of biochemical properties. Furthermore, taking into account of CAS and granular sludge after different treatment or stabilization processes, heavy metals normally presents different availability.

Indeed, sewage sludge must be treated and ‘stabilized’ before any actions to reduce odor, pathogen content and vector attraction before any activity for reusing sewage sludge. They are usually mechanically dewatered or air dried to reduce bulk, to generate a product acceptable for beneficial use. Sludge treatment may be through either one or a combination of the following generic methods:

- Biological process (anaerobic/aerobic digestion, composting)
- Chemical process (lime treatment)
- Physical processes (pasteurization, thermal hydrolysis, thermal drying, air/solar drying)

Mesophilic anaerobic digestion is extensively used by the water industry for the treatment of sewage sludge and is particularly favored due to the production of renewable energy in the form of biomass producing methane. Other methods, as lime treatment or heating or drying at high temperature, provide different quality of sludge, in term of biological composition, structure and matter content. There is a diverse range of process options for sewage sludge treatment and understanding the extent to which they influence not only the nutrient content, availability and fertilizer value but also the heavy metal availability on sludge treatment.

Hence, it is big interest for comparison between conventional activated sludge systems and granular sludge on heavy metal biosorption processes, to understand the key constituents and feature effect of sludge characteristics as well as sludge treatment processes responsible for binding heavy metals. Consequently, it seems particularly important to better predict the future of heavy metals not only following wastewater treatment technologies but also indicating the fate of heavy metal between the water lines and the sludge lines at the stabilization station, and more particularly in the sludge lines. Therefore, the transfer of heavy metals can be carried out when the sludge is disposed.

Currently, the traditional disposal routes for sewage sludge are land application, incineration and landfill. However, both incineration and landfill are hampered by their poor public image, moreover, incineration is both expensive and it gives rise to significant quantities of potentially hazardous ash. Disposal by landfill is further inhibited by its rising cost and the competing pressures for an ever diminishing supply of suitable landfill sites. As the most advantage method, land application of sewage sludge can recycle the nutrient elements (such as N and P) as well as increase soil carbon sequestration (Thangarajan et al., 2013a). The sludge land spreading brings many advantages for soils such as 1) more organic carbon; 2) more available nitrogen, phosphorus and potassium; 3) more crop production; 4) better physical, chemical and biological properties; 5) more available water content and 6) less erosion of soils. Thus, land application



of sewage sludge is both environmentally and economically advisable to improve many physical and chemical properties of soil. Nowadays, sewage sludge has been widely applied to soil in many countries, especially in France. In EU-27, 21 Member States have adopted agricultural reuse, while 53% of the total produced sludge is recycled in agriculture directly or after composting (Kelessidis and Stasinakis, 2012). In France, approximate 75% sewage sludge is applied on land after stabilization. In USA and Canada, more than 50% and 40% respectively, of the produced sludge as bio-solids are applied to land (Citulski and Farahbakhsh, 2010; Venkatesan et al., 2015). In some Asia countries such as China, Korea and Japan, the land application of treated sewage sludge is suggested as the optimal solution for efficient sludge management (Yang et al., 2015). Vietnam lacks efficient wastewater and sludge treatment technologies for pollutant reductions, thus the application of sewage sludge on land has not been developing.

However, heavy metal in sludge can cause problems. High loading of sewage sludge to soil may give rise to an accumulation of heavy metals and the increase of concentration of these metals could lead to phytotoxicity, specific bioavailability, mobility and reactivity in amended soil with sludge. In soil system, metal may constitute part of its solution and/or solid phase. In the solid phase the metals are distributed among the soil components and the association which these components give rise to various physico-chemical forms that determine its mobility. As consequence, it is necessary to establish the legislation or regulation for standard heavy metal content in sludge application on soil, to prevent or minimize hazardous heavy metals residues. The USA, the EU and several countries have set limit heavy metal concentration values and have suggested practices of sludge application rate to prevent harmful effects on soil, vegetation, animals and human. In France, legislation of landspreading permits sludge application rate with 3 tons DS ha⁻¹ year⁻¹ and maximum copper concentration is 1000 mg Cu/kg DS. However, in developing countries like Vietnam, there is lack information of sludge stabilization, therefore, also limit legislation of sludge recycling as fertilization in land spread and heavy metal concerns. Hence, it is important to take into consideration not only the kind of wastewater treatment but also the way of sludge valorization, then sludge application on land, especially in big towns where extensive processes cannot be implemented.

In overview, regard to the study of the influence of sludge treatment processes on the content and behavior of heavy metal in sludge and sludge application particularly on French and Vietnamese soil, recent reports make it possible to have the observation inside the level of knowledge concerning . The bibliometric study of articles in scientific journals (Figure 1) shows in particular that the number of publications dealing with the problem of the fate of heavy metal on sludge (CAS and granular sludge) and on sludge land spreading, has become more and more important recently, since about 3 or 4 years. Granular sludge, since the development in recent decays of this new technology, the numbers of annual papers studying the fate of heavy metal on granular sludge are around 20% of researches on activated sludge for heavy metal behaviors. Besides, there are limited publications concerning heavy metal availability in granular sludge amended soils, especially in case of copper (compared to conventional activated sludge). That makes difficult to get specific statistics which focus on heavy metal availability on granular sludge amended soil.



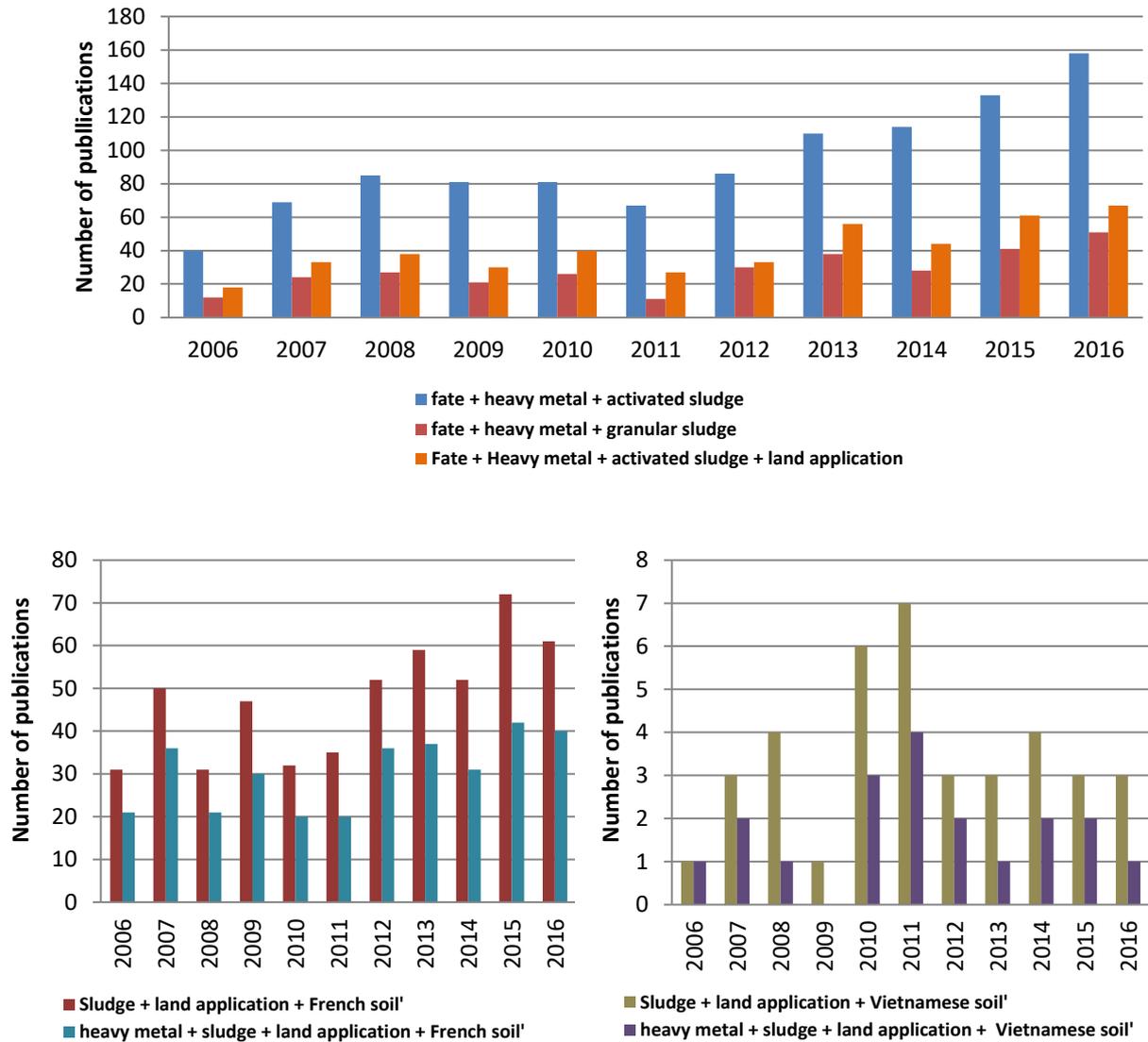


Figure 1: Bibliometric determining the evolution of the number of publications listed in “Sciences Direct” by keywords related to the fate of heavy metal in sludge (CAS and granular sludge) and land application of sludge in French and in Vietnamese soils.

Furthermore, the sewage sludge has been applied for agricultural lands since last 30 years in France leading more concern on studies of sludge application on French soil, especially, more than 50% of this number focused on the behavior of heavy metals. Moreover, the number of published research on the application of sludge and the fate of heavy metals following the spreading of sludge on Vietnamese soil is very rare. The bibliometric study shows the lack of knowledge or scientific information in sludge treatment stages and sludge fertilizing on Vietnamese soil, which may mainly due to the poor wastewater treatment, and sludge treatment technologies. It is therefore a desire to bring new information and a better understanding of the mechanisms involved in the study of the heavy metal behavior and bio-availability according to wastewater technologies (CAS and granular sludge), and stage of sludge treatment (digestion, liming, thickening, drying and composting) before and after spreading. Besides, the comparison the fate of heavy metal, in case of copper, between sludge amended French and Vietnamese soil will provide more information about the effect of sludge on



heavy metal leaching ability when sludges are using like fertilizing in soils according to several conditions of different countries such as human activities, weather condition, etc.

This thesis focuses on the comparison between the granular and conventional activated sludge for the future of heavy metals elements, particularly copper, following stage of sludge stabilization before and after sludge application on one type of French and Vietnamese soils. The ultimate goal is to investigate the effect of sludge and soil characteristics on copper leaching ability and therefore predict the risk as well as potential of heavy metal ability during sludge application on agricultural activities.

The first part of this manuscript (part 1) is devoted to the literature review, in which, the general operation of wastewater treatment and the characteristic of conventional activated sludge and granular sludge (structures, composition) are described. Then, the fate of heavy metal on conventional activated sludge and granular sludge were compared by focusing on the different interactions between heavy metal and sludge. The final part of bibliographic part depicts the impact of sludge and soil characteristics on heavy metal leaching ability after landspreading. The state of the art done in this part shows that sludge characteristics according to wastewater treatment and sludge stabilization method generate impact on the behavior of heavy metals and soil characteristics within sludge soil application.

The different experimental designs and analyzes developed and used to conduct these investigations are presented in the second part (material and methods).

The third part of this thesis describe the results obtained from the application of this approach to the study the fate of copper on CAS and granular sludge before and after land spreading on grass and on paddy soil. In order to achieve understanding of these phenomena, a methodology combining studies conducted in batch and column test were developed. It is synthesized in Figure 2, other schemas that will be taken up later during the presentation of results.

The presented approach has thus consisted, first, in the determination of CAS and granular sludge characteristics. The results are presented in Chapter 1. In Chapter 2, a methodology for investigation of copper adsorption/desorption ability in sludge (CAS and granular sludge), soil and sludge amended soil, has been developed and studied by batch test method. Finally, the copper leaching ability on sludge amended soils were illustrated by column methods, thus, the effect of sludge and soil characteristic to the fate of heavy metal during land spreading was determined (Chapter 3).

The conclusion summaries stayed at the end of the manuscript results. The fate of copper link with sludge and soil properties will be reported. The significant of these findings is also discussed and the main perspectives are also opened by this work.



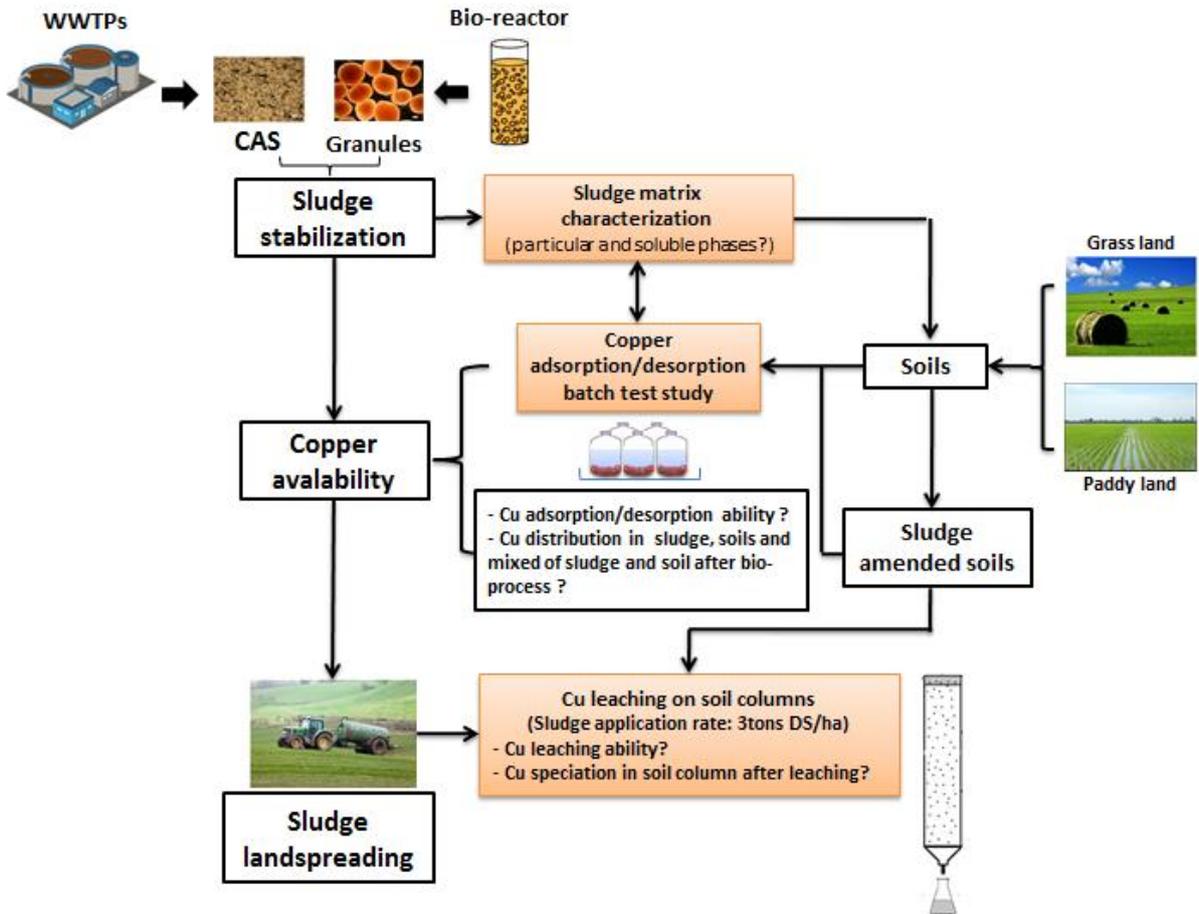


Figure 2: The methodology and manuscript for studying the bio-availability of Cu on sludge and sludge landspreading.



Part 1 : BIBLIOGRAPHIC SYNTHESIS





Part 1: BIBLIOGRAPHIC SYNTHESIS

I. Sludge on wastewater treatment: Processes and performance

Wastewater treatment plants (WWTP), combination of mechanical, physical, chemical and biological processes, are used to achieve pollutants removal from the incoming wastewater. These pollutants are various, and in the case of some wastewaters, may require specific treatment strategies/techniques. In such WWTPs, a primary treatment ensures the separation of particulate pollutants (debris, sand, oils, grease and particular wastes), in particular, thanks to gravitational separation in large settling tank (referred to as primary settler). However, colloidal and dissolved wastes are not sufficiently removed by these separation processes, and therefore, must be eliminated by a secondary treatment stage. In the case of sewage, characterized by a low COD or BOD, biological secondary simple operation and low cost, the activated sludge process is by far the most widely used for sewage biological secondary treatment. Conventional activated sludge is the most popular biological process using in secondary treatment, but attached biofilm on support are used since a long time with classical processes (fixed bed), innovative processes (moving bed biofilm reactor) or emerging processes (granular sludge). This last process is one of the objectives of this study.

I.1. Some data on conventional activated sludge

As generally known, the activated sludge process was invented in England at the beginning of 20th century. At that time, England was a country with urgent problems in water pollution because of dense population and advanced industry. The effort of English engineers, chemists and microbiologists to improve the existing treatment techniques led finally to the invention of activated sludge process in 1914 (IWA – Germany, 2014).

Activated sludge treatment has been used worldwide in secondary treatment system in municipal (WWTP) and industrial wastewater treatment plants. The wastewater purification performances of activated sludge treatment depend heavily on the metabolism and interactions of the microbial community, with bacteria playing a key role in the purification process. Previous efforts have enabled efficient removal not only of suspended solids (SS) and biochemical oxygen demand (BOD), but also of nitrogen, phosphorus and some xenobiotic compounds, via the appropriate design and operation of treatment systems. Nevertheless, activated sludge treatment is still subject to crucial problems, such as unsuccessful sludge settlement associated with sludge bulking and foaming caused by non-floc forming or filamentous microorganism (Martins et al., 2004; Reyes et al., 2002; Wagner et al., 2002). Besides, these systems generally require large surface areas for treatment and biomass separation units due to generally poor settling properties of the activated sludge (A. Giesen et al, 2012). Different units and recycling are required to perform the aerobic, anoxic and anaerobic conversions.



I.1.1. Structure of bacterial flocs

Activated sludge is in form of aggregates of microorganisms, suspended solids, and extracellular polymers. Flocs as microbiological units play an essential role in activated sludge treatment process (Wells et al., 2011). The activated sludge used process takes place as a result of the network formation of EPS excreted by floc-forming bacteria in which the bacteria and other organic and inorganic particles are embedded, and of the filament from filamentous bacteria that serve as a backbone of the microbial floc (Perez et al., 2006). Moreover, Basuvaraj et al., (2015) shown that the morphological appearance of floc grown under non-filamentous bulking condition. The flocs were relatively small (60 – 88 % of the floc consisted of small flocs (<50 um diameter)), irregular, weak, with an open floc structure and containing low numbers of filamentous bacteria. It appears that the viscous bulking observed was caused by an excess amount of EPS in the small flocs. Higher content of EPS leading to weak and small flocs and EPS could contribute to high water retention. EPS can be attached to the cell surface as peripheral capsules that is tightly bound (TB-EPS) or shed into the surrounding environment as a less organized (amorphous) slime, or loosely bound EPS (LB-EPS) (Lin et al., 2014).

Originally, EPS are high molecular weight polymers produced by microbial metabolism and may originate from bacterial secretion, hydrolysis products, ions adsorbed from wastewater and organics adsorbed on the flocs in the wastewater. The total mass of EPS reaches 80% of the total sludge mass (Chen et al., 2015). EPS content in flocs was found to greatly affect surface charge, floc stability and floc size (Mikkelsen and Keiding, 2002). Many studies demonstrated that sludge settling, bio flocculation and dewatering properties have a great relationship with the contents of EPS and the spatial distribution of ESP (Peng et al, 2014; P. Zhang et al., 2014; W. Zhang et al., 2014). The ratio of protein (PN) to polysaccharides (PS) is well correlated with sludge settling and high PN/PS ratio also has significant effects on sludge settling and dewatering. Almost studies found that proteins account for the largest components of EPS with up to 60% (Frølund et al., 1996; Wilén et al., 2003) (Figure 3).

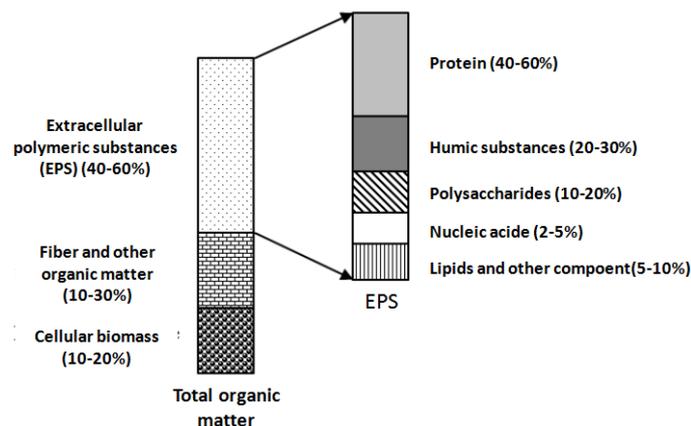


Figure 3: Composition of the organic matter part of the sludge (including extracellular polymeric substances) (Bitton, 2005)

The EPS components carry ionizable functional groups such as carboxyl, phosphoric, and hydroxyl groups. These functional groups like carboxyl would be dissociated, rendering EPSs negatively charged at near neutral pH. The significance of dissociation is controlled by the dissociation constant



of functional groups and pH condition. Surface charged properties of EPS will effect to the interaction of metal ions with surface flocs.

Bacterial distribution in flocs is heterogeneous. For example, the distribution of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) was observed to be stratified in activated sludge. Microbial distribution is related to their resident environment inside activated sludge (Wilén et al., 2008). The relative contents of ammonia-oxidizing bacteria (AOB) and nitrite – oxidizing bacteria (NOB) in flocs population are distributed in two particle size levels (<61 μm) and (97 – 160 μm). The sharp decrease AOB and NOB was observed from 20 – 35 μm below the floc surface, and AOB and NOB were not detected in the floc core. When the particle size of the floc was below 61 μm , both NOB and AOB were distributed evenly from the surface to the floc core and their intensities remained consistent (Basuvaraj et al., 2015). Regarding to phosphorus removal by biological activated process, phosphate accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs) are diverse in floc structures. Therefore, depending on various factors such as type of wastewater and treatment processes, etc., the microorganism distribution in activated sludge can take different places. For instance, in conventional wastewater treatment plants with biomass growing in flocs, different units and recycling are required to perform the aerobic and anaerobic conversion, which lead to arrange the diverse of bacterial in sludge. Figure 4 shows the example structure of flocs in wastewater treatment by activated sludge.

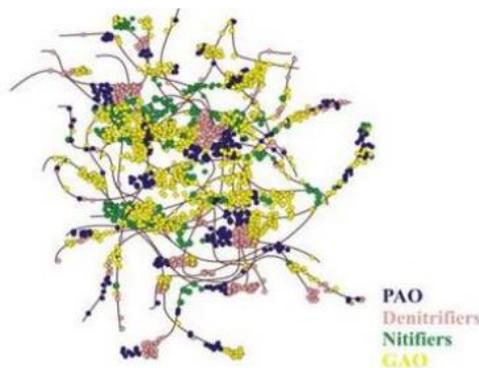


Figure 4: Floc structures of sludge (Castro-Barros, 2013).

1.1.2. Conventional activated sludge process

The conventional activated sludge process (AS) is the most commonly used process for wastewater treatment. Improving its performance is necessary from economic and environmental point of views.

The principles of CAS are:

- A single bioreactor operating continuously where suspended microorganisms consume the colloidal and dissolved organic matter. The reactor is aerated to provide oxygen for aerobic nitrification and biodegradation. Bacteria consume one part of the colloidal and dissolved carbonaceous compounds to satisfy their energetic needs (catabolism), and synthesize another part along with a small proportion of ammonium and phosphorus-into new cellular tissues (anabolism).



- A settling tank (referred to as secondary settled or clarifier) where activated sludge (flocculated biomass) is gravitationally separated from the treated wastewater. The effluent overflows into the receiving waterbody, but in some WWTPs, it may undergo additional treatment (e.g., filtration and disinfection) before being discharged. A minimum solids retention time is required for bacteria to aggregate into flocs (bio-flocculation) so as to enable their gravitational separation.
- The sludge recycles line returning the major proportion of the settled sludge to the bioreactor, thus allowing maintaining a high bacterial concentration in the reactor so as to intensify the biological nutrient removal. A first benefit of sludge recycling is decoupling the hydraulic retention time (HRT) and sludge retention time (SRT), hence, an efficient treatment can be achieved for a hydraulic retention time of the order of ten hours.
- A sludge wastage line at the bottom of the clarifier, from where a small fraction of sludge is withdrawn in order to stabilize the biomass concentration in the bioreactor and to adjust an adequate SRT. The excess sludge withdrawn is then treated separately.

The basic conventional activated sludge allows an efficient removal of carbonaceous organics and ammonium/ammonia from wastewater. However, due to the increasing concern about eutrophication, stricter regulations forced its upgrading to improve nitrogen and phosphorus removal.

Concerning the enhanced nitrogen removal, the two-step nitrification-denitrification process revealed to be the most economical abatement strategy. Nitrification is carried out by nitrifying autotrophic bacteria under aerobic conditions, and consists in the oxidation into nitrate/nitrite. Denitrification is the process of nitrate/nitrite reduction into nitrogen gas, and is achieved by some heterotrophic bacteria under anoxic condition, i.e., DO depletion but nitrate/nitrite presence as a substitute terminal electron acceptor. Carbonaceous matter is consumed by bacteria under both aerobic and anoxic conditions.

In the large-size WWTPs, anoxic and aerobic conditions are carried out in separate reactors. The pre-denitrification and the post-denitrification configurations are the most widespread. In the pre-denitrification design, the anoxic tanks are placed upstream of the aerobic reactors. Therefore, internal recirculation of the nitrified liquor to the anoxic reactor is needed, which involves additional pumping costs. In the post denitrification configuration, the anoxic tank placed downstream of the aerobic reactors. This configuration allows better nitrate/nitrite removal efficiency than the pre-denitrification process. However, it is possible that denitrification continues to occur within the settle, and hinders sludge settling due to the generation of nitrogen bubbles. Moreover, since carbonaceous substrates are consumed in the aerated tanks, they may become limiting in the anoxic reactors. Therefore, the addition of carbonaceous matter (methanol, acetate, and ethanol are the most commonly used) may be required in order to achieve an efficient denitrification.

Concerning phosphorus, the conventional activated sludge leads to removal efficiency around 10-30%. Enhanced phosphorus removal can be achieved by chemical precipitation, or by exposing the biomass to alternating anaerobic and aerobic condition, which intensifies phosphorus uptake by some



microorganisms. The most common configuration enabling enhanced nitrogen and phosphorus removal in the A2O process which consists an anaerobic tanks followed by an anoxic and an aerobic reactor.

I.2. Granular sludge and processes

Granular sludge has become a leading wastewater technology due to its unique features and such as excellent settleability; high biomass retention and high resilience against variation in pollution load (Moy et al., 2002; Tay et al., 2001) and environmental conditions such as temperature and pH. It presents an attractive, low-cost and low-footprint alternative to the conventional flocculent activated sludge process for both municipal and industrial wastewater treatment (de Kreuk and van Loosdrecht, 2006; Yang et al., 2008). To date, granular sludge processes has been implemented in treatment of both municipal and a wide range of industrial wastewaters (de Kreuk and van Loosdrecht, 2006; Adav and Lee, 2008).

Additionally, many reactions can take place in granular sludge by the diverse microorganism distributed in different layer: anaerobic and aerobic reactions can occur at the same granule, since the stratification allows different conditions along the biomass (Gao et al., 2010).

I.2.1. General model mechanism for microbial granulation

Microbial adhesion or self-immobilization is the starting point of granulation process, and can be defined in terms of the energy involved in the interaction of bacterium-to-bacterium or bacterium-to-solid surface. When bacterium approaches another, the interaction between them includes repulsive electrostatic force, attractive van de Waals force, and repulsive hydration interaction. Based on the thermodynamics, some physico-chemical model mechanisms for granulation have been developed; those include inert nuclei model, selection pressure model, multivalent positive ion-bonding model, local dehydration and hydrophobic interaction model and surface tension model.

- **Inert nuclei model**

In the presence of inert micro-particles in reactor, bacteria could attach to the particle surfaces to form an initial biofilm, namely embryonic granules. The mature granules can be further developed through the growth of these attached bacteria under given operation conditions. Figure 5 shows the inert nuclei model which suggests that the presence of nuclei or micro-size bio-carrier for bacterial attachment is a first step towards granulation.

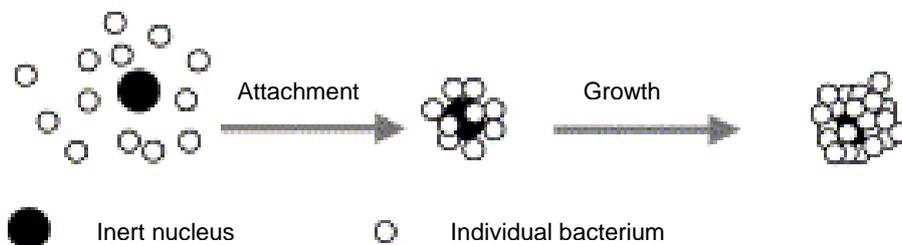


Figure 5: Scheme for inert nuclei model (Liu et al., 2003a).



- **Selection pressure model**

Light and dispersed sludge would be washed out, while heavier components remain in the system. Microbial aggregation in reactor may be effective protection strategies against high selection pressures. Absence of granulation was observed when the hydraulic selection pressure was very weak. Granulation enhanced with high hydraulic stress and short settling time (D. Gao et al., 2010).

- **Multi-valence positive ion-bonding model**

Bacteria have negative charged surfaces under usual pH condition; a basic idea to expedite granulation process is to reduce electrostatic repulsion between negatively charged bacteria by introducing multi-valence positive ion, such as calcium, ferric, aluminum or magnesium ions, into seed sludge (Yu et al., 2001).

The multi-valence positive ions may promote sludge granulation by bonding with extracellular polymers (EPS) (Figure 6). The high affinity between EPS and calcium create the bridge between EPS and EPS and link cells and EPS together to form an initial three dimension structure of microbial community, in which bacteria can further grow.

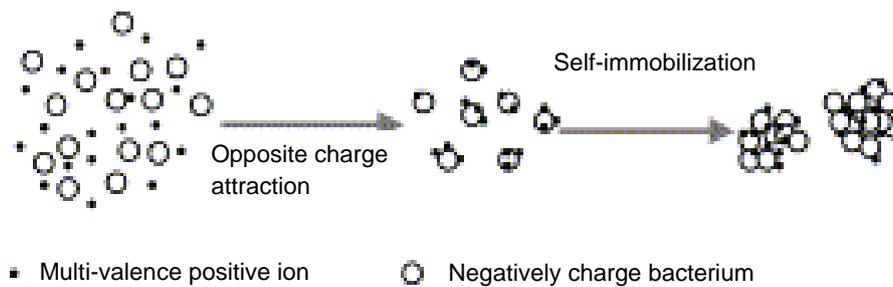


Figure 6: Schematic presents multi-valence positive ion-bonding model (Liu et al, 2003a).

- **EPS bonding model**

The EPS can mediate both cohesion and adhesion of cell. EPS could change the surface negative charges of bacteria, and thereby bridge two neighbor cells physically to each other as well as with other inert particulate matter (Figure 7).

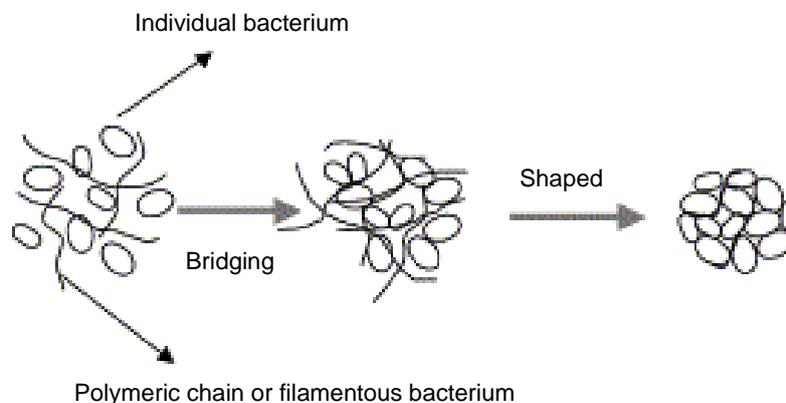


Figure 7: Schematic presents EPS bonding model (Liu et al, 2003a).



EPS bind with the cells through complex interactions to form a net-like structure with plenty of water that protects cells against dewatering (Sheng et al., 2010a), make protective barrier for the bacterial community which contributes to long-term stability (Jiang and Liu, 2012). Interesting, for microbial communication or aggregation-disaggregation hypothesis, EPS has an important role in aerobic granulation. EPS increase the cohesion in the initial phenomenon explaining the granule formation concomitantly with bacterial aggregates densification. Cohesion is a physical property of the aggregate defining how it can resist to shear and elongation forces, which induce erosion and/or break up (Wan et al., 2011). When the cohesion increases, the dense clustering of microbes grows up due to specific interactions of the EPS composing the granule matrix.

EPS are secreted by microorganisms in consumption of organic matters in wastewater and consist of high molecular weight polysaccharides, proteins, glycoprotein, nucleic acids, phospholipids and humic acid (Wei et al., 2012). Both proteins and specific polysaccharides demonstrate granule cohesion. The contribution of anionic proteins, polysaccharides and multivalent cations in EPS aggregation clarified the biochemical nature of the polymers and the interactions involved in the cohesive structure of aerobic granules. The protein-polysaccharide interactions, hydrogen bonding and ionic interaction effectively change the sludge surface properties and are closely related to the formation of microbial aggregates. In addition, EPS can also work together with the cells to establish a special network structure to favor the biofilm formation (Liu et al., 2010a).

EPS is a class of macromolecular material secreted from the cells, and play a key role in the formation of microbial aggregates to create aerobic granules (Sheng et al., 2010a). PN (exo-polymeric protein) and PS (lower polysaccharides) are main components containing large amounts of functional groups such as hydroxyl and electronegative carboxyl (Gao et al., 2010). The different properties of EPS are listed in the Table 1.



Table 1: Main effect of EPS component to granulation

EPS Components	% in EPS	Component properties	Remarks	References
Polysaccharides (PS)	42.7 - 64.6%	PS in EPSs enabled biological groups to form crossed reticular structures through bridging action, combined with the filamentous bacteria twined on the granule surface to form granular sludge	PS belong to the hydrophilic substances	Zhu et al., (2012b). Yan et al., (2015)
Protein (PN)	45.8 - 51.3%	Adhesion; aggregation of bacterial cells; retention of water; sorption of organic and inorganic compounds, binding of enzymes, electron donor or acceptor, and protective barrier to cells	PN belong to hydrophobic substances	Lv et al., (2014) Long et al.,(2014)
Nucleic acids	Less than 10%	Exchange of genetic information, export of cell components	The role depending upon the quantity and available components	Xiao-ying Zheng et al., (2013)
Lipids		Export of cell components	Major to minor role in bio-sorption	J.-F. Gao et al., (2011)
Humic substances	10 -15%	Electrons donor or acceptor	Minor role in the bio-sorption, the impact of humic substances mainly depends the nature of humic substances	J.-F. Gao et al., (2011)

- **Local dehydration and hydrophobic interaction model**

Under normal culture pH condition, the outer surfaces of bacteria are hydrated. Such a water layer on the surfaces of bacteria would prevent one bacterium to approach another. Under physiological conditions, strong hydration repulsion is the main force keeping the cells apart, thus local dehydration of the short distance-apart surfaces would be the prerequisite for cell-to-cell aggregation. Because of strongly hydrophobic surfaces, irreversible adhesion will occur. Increase in the hydrophobicity of cells surface, which in turn to promote cell-to-cell interaction and further serves as driving force for cell self-separation from liquid phase (Liu et al., 2003a).

- **Surface tension model**

Microbial granulation is a process to create a new interface, granule-liquid disrupting preexisting individual bacteria-liquid interface. If the surface free energy of bacteria is lower than the liquid, the free energy of aggregation decrease and aggregation is favored with decreasing surface energy of the inner carrier. The opposite trend would occur if the surface energy of bacteria is higher than the liquid. The aggregation of hydrophilic cells was enhanced at low liquid surface tension, while the opposite was true for hydrophobic cells (Liu et al., 2003a).



- **Cell –to-cell communication model**

Intercellular communication and multicellular coordination have been known as an effective way for bacteria to achieve an organized spatial structure. It has been shown that quorum sensing is a prominent example of social behavior in bacteria, as signal exchange among individual cells allows the entire population to choose an optimal way of interaction with the environment (Liu et al., 2003a).

1.2.2. Aerobic granular sludge formation

Aerobic granules were first reported in early 1990s in an aerobic upflow sludge blanket (UASB) reactor, linked to an oxygen dissolving tank (Mishima and Nakamura, 1991; Shin et al., 1992). In 1997, Morgenroth et al reported the cultivation of aerobic granules in a sequencing batch reactor (SBR). The aerobic granule was defined as follows: “Granules making up aerobic granular activated sludge are to be understood as aggregates of microbial origin, which do not coagulate under reduced hydrodynamic shear and which settle significantly faster than activated sludge flocs” (Morgenroth et al., 1997a). Aerobic granules could be formed in much short time, from 2 to 8 months and have some advantages (Liu et al., 2004), an increasing number of researchers focused on developing technology of aerobic granules for the wastewater treatment since 1997 (Liu and Tay, 2008).

As a promising approach to biological wastewater treatment, the aerobic granular sludge has been proposed. Thanks to their dense structure, aerobic granules have very good settling ability that allows high biomass retention in the bioreactor. This enables the process to withstand high-strength wastewater and results in the biological reactor having a smaller volume than conventional activated sludge systems (Kreuk et al., 2007). The size and density of the granules allow simultaneous nitrification, denitrification and phosphorus removal, SNDPR to be maintained (de Kreuk et al., 2005; Yilmaz et al., 2008a). However, the operating conditions that improve the stability of the reactor’s performance and the physical properties of aerobic granular sludge still need consideration (Filali et al., 2012).

The application of aerobic granular sludge to wastewater treatment may allow removing organic matter, nutrient and some toxic molecules. Aerobic granules contain specific environmental microorganisms, which can remove ammonium from wastewater during simultaneous nitrification and denitrification. Nitrification concerns the oxidation of ammonium to nitrite (nitritation) and then to nitrate (nitrataion) under aerobic condition. During denitrification, nitrate and/or nitrite is reduced to nitrogen gas by heterotrophic microorganism under anoxic condition. Furthermore, new processes for nitrogen treatment are proposed (anammox): ammonium is combined with nitrite to yield nitrogen gas in absence of carbon source (Osaka et al., 2012). Thus, depending on kind of wastewater treatment and operational conditions, the microbial population on aerobic granular sludge is different.

1.2.2.1 Structure and morphology

The shape of the granules is nearly spherical with a very clear outline (Zhu and Wilderer, 2003). The average diameter of aerobic granules varies in the range of 0.2 to 5 mm. This is mainly due to operational parameters, such as seed sludge property, substrate composition, organic loading rate,



feeding strategy, reactor design, and hydrodynamic shear force. The color of aerobic granules is usually yellow (Figure 8) (Liu et al., 2009a).

The collected aerobic granular sludge was smooth on surface appearance and intact in structure. The internal core was white with crowded spots. Moving outward the color turned yellow, suggesting the presence of excess biomass. The interior of the granule was compact with layered structure (Lv et al., 2014).

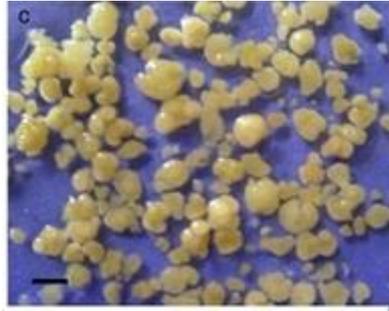


Figure 8: Images of seed sludge and mature aerobic granules (Bar = 5 mm) (Li et al, 2008).

Sheng et al., (2006) established a multi-layer structural model to describe the aerobic granular structure with two distinct structural regions for the microbial aggregates. The outer part contains dispersible cells loosely entangled by the readily-extractable EPS fraction. The inner part contains biomass in a stable structure tightly glued by EPS, which could not be dispersed by shear except under unfavorable microbial aggregates. Adav et al., (2008) reported that microbial granule structure was stabilized by a network principally composed of β – polysaccharides as the backbone for embedded proteins, lipids, α – polysaccharides, and cells. In native granules, glucose or mannose-containing carbohydrates stained by Con A and protein stained by FITC. Caudan et al., (2014) found that carbohydrate and protein have similar localization within the large crown surrounding the core (made of precipitated mineral compounds). Proteins were distributed in all the structure while glucans appeared with slightly higher density at the external periphery of the granules (Figure 9).

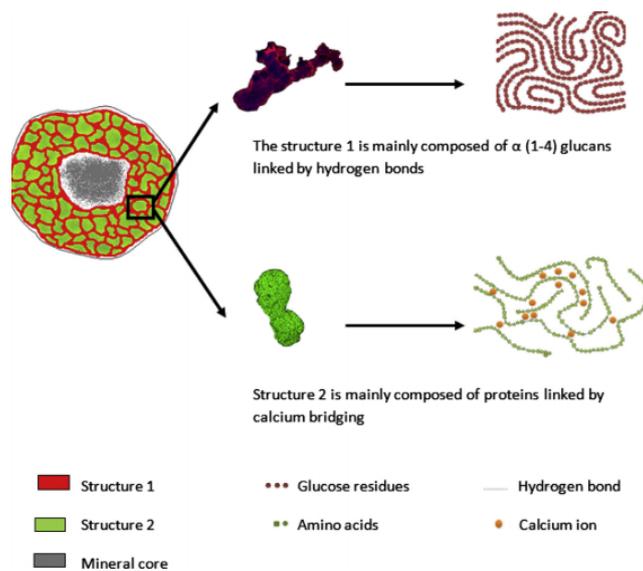


Figure 9: The model structures of aerobic granular sludge (Caudan et al, 2014)



I.2.2.2 Technology for granulation and wastewater treatment

Aerobic granulation has been mainly achieved in SBR and strongly related to operating conditions, which should be favorable for microorganism to form aggregates and/or granular particles. Sequencing batch reactor (SBR) process is known to have several advantages over conventional continuous flow system. In recent years, the use of single tank SBRs for the biological treatment of wastewater has been widely extended from lab-scale studies SBRs usually operate with fixed times for the different phases of filling, mixing (anaerobic, aerobic, anoxic or complex between these conditions), settling and decanting due to influent fluctuation and system state variations. Higher levels of process control and automation are necessary to optimize the SBR operation (Lemaire et al., 2008). Recently, a number of SBR technologies have also been developed for biological nutrient removal from many different kinds of wastewater. Table 2 presents several type of wastewater which was treated by aerobic granules in SBR – reactor.

Table 2: Process efficiencies of Aerobic Granular Sludge - SBR for wastewater treatment

Reactor type	Wastewater type	Kind of organic matter treated	Process efficiency (%)	Timing treatment (days)	References
SBR (pilot-scale)	Real wastewater	COD and N removal	80 (COD); 92 (N)	400	Y.-Q. Liu et al., (2010a)
SBR (lab-scale)	Domestic wastewater	Carbon and BNR	95,7 (C); 84 (N); 92 (P)	14	Verawaty et al., (2010)
	Abattoir wastewater	COD and BNR	88,4 (COD); 89,7 (N); 2 (P)	18 - 133	Pijuan et al., (2011)
	Real dyeing textile wastewater	COD and N removal	62 – 78 (COD); 80 – 90 (N)	36	Lotito et al., (2012)
	Municipal wastewater	COD and N, P removal	> 90 (N, COD, P)	104	Bassin et al., (2012)
	Rubber wastewater	COD and N removal	96,5 (COD); 89,4 – 94,7 (N)	90	Rosman et al., (2013)

- **Effecting factors**

To date, published results indicate many operational factors have significant influence on granulation process in SBR.

- **Seed sludge or sludge inoculums**

In most studies, aerobic granules were cultivated with activated sludge since the bacterial community that resided in this environment was important for the aerobic granulation process (Lee et al., 2010). Flocculent sludge is believed to be the precursor of aerobic sludge granules. According to the recommendation of granulation from the microbial communication and/or aggregation, seed sludge



keeps the role as a substrate in the initial granulation process. It serves to promote the process of creating rapid or slow particles. Pijuan et al., (2011) found that the granulation time strongly depend on the fraction of the crushed granules with higher fraction leading to faster granulation. The interaction between crushed granules and floccules sludge during granulation and their contribution to the faster remain to be elucidated. Using crushed granules instead of intact granules has a positive effect on reducing the start-up time. With other ideas, Song et al., (2010) created 2 SBRs reactor: one reactor was inoculated using fresh activated sludge taken from beer wastewater treatment plant, the other reactor was inoculated using fresh activated sludge taken from municipal wastewater treatment plant. More hydrophobicity and microbial community structure changed: a lot of varied quantities of dominant species accelerate the aerobic granulation when aerobic granules were cultivated in beer wastewater compared with activated sludge from municipal wastewater treatment. Zhang et al., (2010) cultivated aerobic granules by seeding completely autotrophic nitrifying sludge in an SBR with 120 – 650 mg/L ammonia under loading rate of 0.48 – 3.6 kg/m³day. These authors cultivated mature 0.5 mm aerobic granules within 43 days cultivation. Liu et al., (2010) cultivated aerobic granules in an SBR using real wastewater as feed and noted that about 400 days were needed to obtain mature granules. Hence, the different group of bacteria, and the composition of hydrophobic counterpart of seed sludge can be the major reason for faster aerobic granulation.

➤ **Feeding**

The operation of nearly all aerobic granular sludge SBR systems comprises four steps: feeding, aeration, settling and discharge. In most cases, feeding time is often as short as a few minutes. This diminishes substrate gradients in the liquid phase, to favor the growth of non-filamentous bacteria. There is evidence that adding substrate in different aerobic feeding periods could create strong substrate gradients in SBR and, consequently, improve the settle ability of sludge (Am et al., 2003; McSwain et al., 2004). According to these researchers, feeding patterns create favorable conditions for the development of non-filamentous bacteria that high substrate uptake rates during periods of high substrate concentration and a capacity to store reserve materials during periods of starvation (Liu and Liu, 2006; Martins et al., 2004).

By contrast but interestingly, the studies of Winkler et al., (2012) and Lotti et al., (2014) aimed at demonstrating on lab-scale the feasibility of granular sludge-anammox based on single stage (aerated system with both nitrification and anammox bacteria coexist) autotrophic nitrogen removal at low temperature. The feeding times represented about more than 30% of cycle time. For these purposes, during the anoxic feeding period (60 min) nitrate produced during the previous cycle was mixed with medium containing acetate and ammonium.

Wang et al., (2012) compared the granulation process of two SBRs: for one reactor, wastewater was fed in one batch at the start of each cycle, in the other reactor, half of the wastewater was fed at the beginning of each cycle and the other half was fed within the same cycle. They determined that two step feeding had adverse effects of granulation. Feeding included 2 stages: the first stage was fed with high concentration of simulated wastewater to 50% of the effective volume of SBR, then the reactor was anaerobic for 60 min with no stirring, then creating a high substrate concentration area similar to



biological selector; the second stage was filled with tap water for the rest 50%. They obtained granules after only 18 days with irregular, light yellow, and average particle size was 1.59 mm.

Until now, almost studies of granulation operated fast feeding mode. The substrate gradient absorbance between inside and outside layer of granules is believed to be crucial for the granulation process. However, based on the requirements of the effectiveness of sludge particles in wastewater treatment process, more researches are needed to demonstrate the impact of this phase for the granulation and improve the efficiency of wastewater treatment.

➤ **The cycle**

The unique feature of a SBR is its cyclic operation, which leads to the periodical biodegradation phase followed by the aerobic starvation phase in every cycle (Liu and Tay, 2006). The cycle time represents the frequency of solid discharge through the effluent withdrawal or the so-called washout frequency, and it is interrelated to the hydraulic retention time (HRT). When the hydraulic selection pressure in term of HRT is high, microorganisms are either washed out or retained in the reactor through the formation of granular sludge (Wang et al., 2006). Shorter cycle time with more cycle numbers implies stronger hydraulic selection pressure during the certain period, which led to the more frequent biomass washout and faster granulation. Liu and Tay (2007) noted that, as the cycle time increased from 1.5 h to 8 h, the specific biomass growth rate of granular sludge decreased from 0.266 to 0.03 L/day, while the corresponding biomass growth from 0.316 to 0.063 g/g COD. Additionally, the granules cultivated at 1.5 h cycle time were the biggest in size while the granules cultivated at 4h cycle time were the most compact compared with those cultivated at other cycle time. However too short cycle time is unfavorable to the long-term stability of aerobic granules because there is no need to worry about the loss of microbial diversity of aerobic granules

Additionally, in aerobic granular sludge SBR, many stresses can become repetitive with the cyclic operation that is characteristic of SBR. Therefore, to improve efficiency and promote granulation process of wastewater treatment in parallel with maintaining the stability of granular structure in the long-term, the cycle time should be adjusted with other parameters to optimize the treatment process (Lotti et al., 2014; Ni et al., 2008).

➤ **Substrate composition**

Various substrates were used to cultivate aerobic granules. Depending on the kind of wastewater, the granulation formation varies markedly. From synthetic wastewater, aerobic granules have been cultivated with an inorganic carbon source and ammonium and were dominated by nitrifying bacteria (Zhong et al., 2013). Granules cultivated with inorganic carbon and ammonium had dominancy of ammonia oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) (Lotti et al., 2014). Some granules were cultivated with acetate and glucose and propionate as a carbon source (Zeng et al., 2013) and nitrate as a nitrogen source (Chen et al., 2016). Wang and Zhou (2010) cultured aerobic granules from activated sludge by using nitrobenzene as sole carbon and nitrogen source. It has been noted from the literature that the carbon source dictates the diversity and dominancy of the bacterial species,



granule surface and structure. Yang et al., (2014) cultivated aerobic granules from propionate wastewater. Erşan and Erguder (2013) used acetic acid in anoxic/aerobic and aerobic/anoxic condition

Information on granule cultivation with real-like wastewater was also reported such as abattoir wastewater (Yilmaz et al., 2008b), dairy effluent or domestic wastewater (Y. Liu et al., 2010). The real wastewaters are very often characterized by variable compositions due to a change in production intensity of certain products, e.g., seasonal production in food and flavor factories or market driven changes. The substrates loading content high range of compositions fluctuation (Van Dierdonck et al., 2012). Thus, the formation of aerobic granules takes several months. In (Yilmaz et al., 2008a), 170 days were need to adapt aerobic granules developed with synthetic wastewater to abattoir wastewater. In (Ni et al., 2008), 300 days were needed to achieve 85% granulation in a reactor treating domestic wastewater. Zhao et al., (2013) cultivated an aerobic granular sludge with modified piggery wastewater and obtained granules after 40 days with high capacity of nitrogen and COD removal in the role of bacterial diversity of strain and heterotrophic nitrifier microorganisms.

➤ **Organic loading rate**

OLR played an important role in developing and sustaining aerobic granules in the wastewater treatment system. The decrease in COD concentration of wastewater decreases the food to microorganism ratio (F/M), thus most of the biomass in the inner core of granules will face substrate scarcity due to the diffusion limitation. The activities at the inner part of granules may thus be seriously suppressed. Therefore, some of the granules disintegrated into small debris which deteriorated the biomass settling activity and subsequently washed out from the reactor. Under this starvation condition, much smaller granules were formed in the reactor, while the COD performance was not affected. For detail, at low organic loading rate the significant damage to granule structure is the dominant granule morphology with size over 540 μm which was maintained throughout the operation. Aerobic granules at sizes of about 750 μm were finally obtained at the low OLR after long time cultivation (Ma et al., 2013).

When the concentration of COD was high, F/M ratio increased, more aerobic granules are formed and high biomass retention occurred in the reactor (Peyong et al., 2012). With the same opinion, aerobic granules were typically cultivated from the wastewater at high organic loading rate (OLR) (López-Palau et al., 2009). D. Gao et al., (2011) showed that high OLRs enhanced granulation rates. The OLR affects the granulation rate and the microbial communities in formed granules. The rates of granulation had significantly increased from the start-up date. Their results implied that a relative high specific loading rate near 0.4 g COD/(g SS. day) could be favorable for the initial formation of aerobic granules and a higher loading rate applied could stimulate formation of larger granules compare to lower rate.

The development of larger granules at a high loading rate was likely due to deep penetration of substrate inside granules without substrate limitation. Zhang et al., (2013) cultivated granules in less than 24h with an OLR of 24kg/(m³day) and settling time of 1 min. However, the cultivated granules had poor stability. Yang et al., (2014) studied the effect of different OLR to granulation. They operated two ranges COD fed at OLR between 4.4 and 17.4 kg/(m³h). They noted that the shifts of COD fed change from 1500 to 5000 mg/L in 8 days and then suddenly decreasing to 1500 mg/L for 3 days,



and then again increasing from 2500 to 5000 mg/L from day 16th did not significantly, minimally alter the appearance of the formed granules. A further increase in the COD loading led to simultaneously decreases of PN/PS ratio and sludge surface hydrophobicity, while the disintegration of aerobic granules was observed. Aerobic granules formed at different organic loading rates had different morphologies, structural properties and bacterial species (Zhu et al., 2012a).

In order to clarify the effect of high and low OLR to the granulation, Peyong et al., (2012), studied the changes in microbial morphologies and structures of aerobic granules under different organic loading rate. The development of aerobic granules was carried out in 3 stages using SRRs: (1) synthetic wastewater with OLR of 1.2 kg/(m³day); (2) low strength real domestic wastewater with OLR of 0.13 kg/(m³day); and (3) domestic wastewater with added external carbon source of acetate to give an OLR of 0.6 kg/(m³day). In the granulation process, the SVI of biomass in each SBR changed with high SVI value around 165 mL/g with real wastewater and 78- 88 mL/g for synthetic wastewater and domestic added source carbon respectively. They also found that the OLR as low as 0.6 kg/(m³day) was able to develop and maintain the integrity of aerobic granules and real wastewater with more particulate OLR was found to enhance the growth of filamentous and protozoa like microorganism in the granules.

➤ **Dissolved oxygen**

Changing the DO concentration manipulating the air-flow rate may also cause a change in the density of the granules. Theoretically, the depth of DO concentration in the granule depends on the DO concentration in the bulk solution and the oxygen consumption rate of the granules (Liu and Liu, 2006). The dense-spherical structure of granules leads to transfer limitations, promoting the dissolved oxygen gradients and efficiency of denitrification (Di Bella and Torregrossa, 2013). In fact, the simultaneous nitrification-denitrification largely depends on the DO concentration in the bulk liquid and the granular size (Coma et al., 2012). As described by Di Bella and Torregrossa (2013), satisfactory SND was performed even in the high DO concentration (> 7 – 8 mg/L) because of big mean granule size (> 1.5 mm). Yuan and Gao (2010) also reported that the total inorganic N removal were increased when DO concentration were decreased from 4.5 to 1.0 mg/L, and the nitrification via nitrite process was stably achieved in aerobic granular sludge reactor (0.5 – 1.0 mm in diameter under different DO concentrations during the aeration phase).

➤ **Hydraulic shear stress**

Hydraulic shear force has been founded to have a significant impact on the formation of aerobic granules and high shearing force is one of the key factors for aerobic granular formation (Y.-Q. Liu et al., 2010a). Lots of researches showed that aerobic granules could not well developed at a low superficial air gas velocity as 0.008 cm⁻¹, but were observed at higher velocity as 0.025 cm⁻¹ (D. Gao et al., 2010). It is worth noting that the shear force changes the EPS content. When the surface gas velocity reached from 0.5 cm/s to 3.6 cm/s the content of PN increased and PS of EPS decreased. The high shear force causes more frequent collision among granules and/or stronger friction between granules and liquid. The cells loosely grown on the surface are removed and the formation of big granules is prevented. In addition, it is noteworthy that in the higher hydraulic shear condition (surface gas velocity of 3.6 cm/s),



the sludge surface hydrophobicity was also higher, and obvious disintegration of granular sludge was found in the reactors with higher effluent (Zhu et al., 2012b).

However, Wan et al., (2011) indicated that high shear stress were not necessary to produce granules, but probably only accelerate the accumulation of granules. They created the granular sludge at 0.6 cm/s at settling time is 30 min. Low high shear stress and long settling time made the good conditions for the aggregates collision.

➤ **Sludge settling time**

The settling time is another key factor in the sludge granulation with a low-concentration wastewater. In an SBR, wastewater is treated in successive cycles, each of which lasts several hours. At the end of each cycle, the biomass is settled before the effluent is withdrawn. The settling time acts as a major hydraulic selection pressure on sludge. A short settling time preferentially selects for the growth of rapidly settling bio-particles and the bio-particles with poor settle ability is washed out. A lower settling time would result in a higher settling velocity for the retained bio-particles.

The readily retainable bio-particles in the SBR ensures a more rapid and efficient granulation. In many studies, a short settling time has been commonly applied to enhance the aerobic granulation in SBRs. With a long settling time, poorly settling sludge flocs cannot be withdrawn effectively and they may in turn out competence granule-forming bio-particles on nutrients for growth. As a result, aerobic granulation cannot be achieved in an SBR operated at a longer settling time. This suggests that the settling time is a crucial factor in the granulation of activated sludge (Ni et al., 2008). Beside, long settling time was also unfavorable for granulation as it could not efficiently supply biological selection pressure to screen heavy aggregates with good settling ability. Actually, short settling time was the decisive factor responsible for aerobic granulation (Zhang et al., 2013).

In some studies, the very short settling time often setup in the range 2-5 min. A large amount of biomass washed out, but the filamentous bacteria disappeared after only a short time, the aerobic granules appeared more compact and achieved high performance for processing of N and P removal (Lotti et al., 2014; Wan et al., 2014, 2013). Settling time was progressively shortened from 10 to 5 min, which provoked the almost complete wash-out of the inoculated activated sludge and the selection of biomass with better settling properties (Wang et al., 2014). However, although the light flocs could be effectively washed out when the settling time was short, a huge loss of sludge and newly formed aggregates made the biomass per volume decrease seriously as the initial formation of the granules was slow, consequently leading to OLR increment, which might be likely to cause sludge bulking during long operation (Zhang and Zhang, 2013).

In other papers, the settling time is not usually fixed (for example in installation that changes according to the response time). This created stability and development in biomass during granulation. The settling time often decreased from 30 to 5 or 2 min: This suggest that the effects of selective pressure to granulation (Long et al., 2014). On the contrary, Wan et al., (2014, 2013, 2011), Zheng et al., (2013) and Wan et al., (2011) indicated that the settling time did not greatly affect the time for granulation. They keep the long settling time at 30 min during the granulation process.



Hence, although the effect of settling time for the granulation is important, it also depends on many other parameters of the system, like the interaction during granules formation.

➤ **Sludge retention time**

Recently, SRT is recognized to be inversely correlated with the specific microbial growth rate. Long SRT implies a low specific growth rate. During the formation of aerobic granules, a substantial amount of suspended sludge is discharged out of the SBR in accordance with the preset selection pressures in terms of settling time, volume exchange ratio and effluent discharge time (Liu et al., 2005; Zhu et al., 2013). Besides, a long SRT favors filamentous growth because of a low specific growth rate of filamentous bacteria, which lead to very poor settle ability of the granules became and their potential washed out of the reactor (Liu and Tay, 2006). Li et al., (2008) studied the effect of SRT and hydrodynamic selection pressure: they showed that with longer SRT, the mean size of aerobic granules bigger than 350 μm was found to be less than 20% and bio-flocs were dominant form of biomass.

G. Sheng et al., (2010) studied the importance of sludge retention time controlling with excess biomass discharge in bioreactors. This study used a fixed daily sludge discharge ratio of around 10% of slow sludge in granular sludge reactor, and found that this selective sludge discharge facilitated the growth and accumulation of denser sludge in the reactor. Winkler et al., (2011) used the discharge mode on the top of the sludge bed to improve the performance of aerobic granular sludge. Zhu et al., (2013) want to improve the long-term stability of aerobic granular sludge process with a discharge of 5-10% of the total granular sludge in order to achieve an SRT of 9.9 days and obtained that the granular rate and average granular diameter of the bioreactor were stabilized at 80% and 2.3 ± 0.3 mm, respectively.

Long sludge retention time (30 days) played a crucial role in the accumulation of precipitated phosphate in the core of granules (Li et al., 2014). Thus, successful operation of aerobic granular sludge in SBR requires that SRT should be carefully managed to ensure that it is within a range that is generally acceptable for aggregated forming granules creation.

➤ **Exchange ratio**

For an SBR system, the OLR depend on both influent substrate concentration and hydraulic loading rate (HLR). Thus, if a low-concentration wastewater is used as the substrate and a usual HLR is employed, the resulting OLR will not be sufficiently high to keep sludge increase for granulation. On the other hand, an increasing OLR, attributed to an elevated HLR, will compensate for the low substrate concentration, and this in turn in favor of the granulation process. In order to keep a high OLR and accordingly to achieve sludge granulation in a pilot-scale SBR for the treatment of municipal wastewater, its HLR has to be kept at a high level. The HLR of an SBR is governed by its volume exchange ratio (VER) and cycle period. In the work of Ni et al., (2008), the cycle period was set at 3h because of the low total COD level in the wastewater. In this case, keeping a high level of VER was the only way to elevate its HLR. The VER of the pilot-scale SBR was gradually increased from 50 to 70%.



II. Fate of heavy metals during wastewater treatment: comparison between conventional and granular sludge

Heavy metals could be defined as metals with relatively high densities, atomic weights, or atomic numbers. Ever increasing, industrial activity is the main culprit behind most environmental pollution problems and ecosystem damage, due to the accumulation of pollutants such as toxic metals: Chromium (Cr), Copper (Cu), Lead (Pb), Cadmium (Cd), Nickel (Ni), etc., (Pino et al., 2006). Contamination of soils, groundwater, sediments, surface water and air with hazardous and toxic chemicals poses significant problems for both human health and the environmental (Ansari and Malik, 2007). Heavy metals are considered to be particularly dangerous pollutants (Aksu and Açıkel, 2000; AlRub, 2006; Kang et al., 2007). Upon entry to WWTPs, heavy metal may sorb on activated sludge flocs (Semblante et al., 2015).

Activated flocs of microbial biomass can retain relatively high quantities of metals by means of a passive process known as biosorption, which is dependent on the affinity between the metallic species or its ionic forms and the binding sites on the molecular structure of the cellular wall (Ruiz-Manríquez et al., 1998). Binding sites are present in cell wall, composed of lipopolysaccharide, peptidoglycan, and phospholipids and also present in EPS (Yee et al., 2004). In contrast to mineral surfaces, the surfaces of microorganisms contain multiple reactive layers, each with a distinct structure and chemical composition (Al-Asheh and Duvnjak, 1998; Tobin et al., 1990). Hence, heavy metal sorption on sludge largely depends on their physico-chemical properties (Semblante et al., 2015).

Compared to biomass or bioflocs, granular structure – based biosorbent has the advantages of denser microbial structure and more excellent settling ability (Wei et al., 2013). To date, anaerobic granular sludge is widely applied for treating industrial wastewater in full-scale, while aerobic granular sludge technology was developed during the last decades at laboratory scale (Morgenroth et al., 1997b; Tay et al., 2002; Zeng et al., 2003) as well as pilot scale (Y.-Q. Liu et al., 2010b; Morales et al., 2013). Recently, aerobic granular sludge is applied by Royal HaskoningDHV in the Nereda technology which was first adapted for industrial applications and then further scaled-up for domestic sewage treatment (Roest et al., 2011). Few papers have been published so far describing full scale operation of the aerobic granules on domestic wastewater (Giesen et al., 2013). The studies of aerobic and anaerobic granulation indicated that granules possess compact porous structures, excellent settling ability, and high mechanical strength. Even under aggressive chemical environmental (acidic or base conditions) the biomass demonstrated high stability with no visible structural damage (Hawari and Mulligan, 2006a). In addition, aerobic granules, which are used as novel techniques for the biological treatment of wastewaters, constitute special self-aggregated biofilm. The biomass of attachment of aerobic granular sludge was about 300% higher than that of floc activated sludge, which clearly indicate that aerobic granular sludge had a stronger microbial attachment ability and that the microbial attachment of aerobic granular sludge may have an important contribution to the development of heavy metals adsorption abilities in aerobic granular sludge (Hao et al., 2016).



II.1. Concentrations of heavy metals in conventional sludge and in granular sludge

Heavy metals are non-biodegradable compounds that can be found in sewage sludge and thus may influence and be influenced by the predation process. Individual heavy metals showed significant difference in distribution between sludge and the treated effluent in the activated sludge system (Dong et al., 2013a). Rapid urbanization and industrialization have been coupled with a sharp increase in municipal solid waste. Sewage sludge, which is usually generated by wastewater treatment plants, accounts for a major portion of this municipal sewage sludge (Dong et al., 2013a).

The activated sludge, being the most commonly used biological wastewater treatment method in WWTP, has been proved to be one efficient adsorbent to remove and accumulate heavy metals in wastewater. The accumulation of heavy metals occurring during stabilization of sewage sludge is an important aspect that should be considered in order to secure the agricultural land application of dried sludge as a cost-effective and sustainable option. The concentration of HMs in the sludge is found to be nearly 0.5-2% on dry weight basis, which may go up to as high as 6% in some cases. During sewage treatment, about 50 – 80% of the total quantity of HMs present in sewage gets fixed into the sludge by various physico-chemical and biological processes (Caicedo et al., 2015). The conventional sludge treatment operation is mainly aimed at increasing the concentration of total solids in order to reduce the sludge volume (i.e., sludge thickening and dewatering) or decreasing the concentration of volatile solids and stabilizing the biodegradable fraction of organic matter (i.e., sludge stabilization via anaerobic digestion or composting). But conventional sludge drying technologies (like filter processes, centrifuges, etc.) are also very expensive to establish due to their high operation, maintenance and energy consumption costs. Depended on the different stabilization sludge process, the HMs concentrations can increase or decrease (Cestonaro do Amaral et al., 2014).

Granular sludge played a promising role in adsorption of toxic chemicals due to a high surface area, porosity and good settling capability. The highly toxic heavy metals had been removed with sludge granules by biosorption. The maximum adsorption density of aerobic granules was three times greater than that of sludge flocs (Adav et al., 2008b). The concentration of HMs accumulation on granular sludge varied according to the wastewater type and granulation during wastewater treatment process. Aerobic granular sludge was normally created by SBR lab-scale reactor using synthetic wastewater. The content of HMs was often simulated domestic wastewater, the HMs content did not so huge. Thus the HMs concentrations determined on aerobic granular sludge conventionally lower than that on large scale WWTP of conventional sludge.

Table 3 presents several HMs concentrations in sludge. Cu, Cd, Zn, Cr and Pb, are popularly HMs which were found on both of conventional and granular sludge. The HMs concentration depended on wastewater characteristic and sludge formation process. Copper pollution especially arises from industries and excessive use of Cu-based agro-chemicals. So that indicates Cu concentration was often found higher concentration in industrial wastewater than in domestic or urban wastewaters. Besides, Cu along with zinc, arsenic, lead, and mercury, is recognized more stable in dewatering sludge with large contents, which raise the attention for reuse sludge such as landspreading.



Table 3: Several heavy metals concentration on conventional sludge and granular sludge

Sludge	Sludge origin	Heavy metals content (mg/kg)											Refs
		Cu	Zn	Pb	Cd	Cr	Ni	Fe	Mn	As	Mo	Hg	
Anaerobic digested sludge	WWTP (Urban wastewater)	203	821	223	13.3	-	-	-	-	-	-	-	Longmian Wang et al., (2013)
		99.7	473.4	39.53	0.59	6.3	12.1	-	-	-	-	-	Zhang et al., (2012)
		181.7	1453.9	40.3	-	293.7	114.8	-	-	-	-	-	Dong et al., (2013b)
Activated sludge	WWTP (45% industrial and 55% domestic wastewater)	1416	4946	-	-	1668	287	-	-	-	-	-	X. Wang et al., (2015)
Thermal drying activated sludge	WWTP (domestic wastewater)	108.8	1871.9	-	93.6	78.8	-	-	-	-	-	-	Li et al., (2016)
Drying reed bed sludge	WWTP with reed (<i>P. australis</i>) after 12 years	343	1446	43	-	37	20	7830	269	-	11	-	Caicedo et al., (2015)
Dewatered sludge	WWTP (industrial wastewater)	287.9	1362.1	-	-	-	-	-	-	-	-	-	Hei et al., (2016)
Aerobic granular sludge	SBR lab-scale (synthetic wastewater)	-	4939	-	-	-	-	-	-	-	-	-	Marques et al., (2013)
		44.14	0.239	-	84.52	-	21.72	-	-	-	-	-	Xu and Liu, (2008a)
Anaerobic granular sludge	UASB lab-scale (synthetic wastewater)	-	-	0.29	0.008	0.008	-	-	-	0.62	-	1.53	Yu et al., (2016)



II.2. Mechanisms linked to the fate of trace metal element during wastewater treatment

In wastewater treatment processes heavy metal are not degraded, so the fate of heavy metal is only due to their ability to be either in the particulate phase (waste activated sludge) due to bioaccumulation, adsorption, precipitation, either in the “soluble” phase (treated water).

As a consequence, the fate of heavy metals in sludge is due to physical and chemical treatment mechanisms such as sedimentation with suspended solids, co-precipitation by organic compounds and chemical precipitation as well as in microbial process. Heavy metals can be actively bound by living microorganisms by means of the following mechanisms: intracellular accumulation, extracellular precipitation and chemical transformations catalyzed by these microorganisms, such as oxidation, reduction, methylation, and demethylation. Passive mechanisms of metal binding are as follows: extracellular complexation of metal by substances excreted by cells and bio sorption – binding of heavy metals to active groups of chemical compound of cell walls and membranes.

Accumulation and transportation of heavy metals in a sewage treatment plant are governed by various physico-chemical and biological mechanisms (Chipasa, 2003):

- **Bioaccumulation: active interaction between cells and the metal ions which can penetrate inside thereof.**

It is a metabolism – dependent process. Metal sequestering process by living biomass can be characterized by intracellular accumulation, which involves metal binding on intracellular compounds, intra-cellular precipitation, methylation and other mechanisms. Transport of metal across the cell membrane can occur by diffusion across the outer wall, but in conditions of starvation for essential metals also specific transport can be induced for heavy – metal internalization (Ledin, 2000).

- **Biosorption: sum of all passive interactions (not related to the metabolism of cells) between the CEP and the cell surface.**

It is a passive uptake process which is generally fast, mostly reversible and independent from cell viability. Biosorption is then primarily a physico-chemical rather than a biological process based on ion exchange, complexation and surface micro-precipitation reactions involving a large variety of binding sites of extracellular polymeric substances and bacterial cell surface (Guibaud et al., 2005).

- **Sorption on the particulate matter present in the wastewater.**

Suspended particle matter in the wastewater is a complex mixture of inorganic particles and organic material and few attempts have been made using natural suspended particles to access information related to their sorption heavy metals (Grassi et al., 1997).



- **Precipitation: depending on their solubility, the metals will be present in a more or less important in the soluble fraction of the waste water and sludge.**

The solubilized heavy metals can be removed by precipitation on sludge. The formed precipitate is removed from the liquid by a second physical separation step, resulting in a smaller amount of metal-rich sludge compared to the initial huge fraction of sludge. The metals precipitation process could be due to pH variation, and co-precipitation with other chemical species.

To these four major mechanisms should be added the possible metabolic reactions (oxidation, reduction, and methylation) induced by microorganisms that modify the speciation the considered metal element.

II.2.1. Precipitation

The complex structure of sludge implies that there are many ways for the metal sorption. The sorption mechanisms are various and are not fully understood. They may be classified according to various criteria. In case of precipitation, it may be either dependent on the cellular metabolisms of microorganism distribution on sludge or independent of it. In the former case, the metal removal from solution is often associated with active defense system of the microorganisms. They react in the presence of toxic metal producing compounds, which favor the precipitation process. In the case of precipitation not dependent on the cellular metabolisms, it may be a consequence of the chemical between the metal and the cell surface. The various biosorption mechanisms mentioned above can take place simultaneously (Javanbakht et al., 2014).

- **Comparison between CAS and granular sludge**

The pH of sludge often knows as factor impacting HMs precipitation on sludge. In sludge, metal speciation and hence their solubility are significantly affected by the solution chemistry, especially pH (Dong et al., 2013c; Singh and Kalamdhad, 2012). With increasing pH, most metal ions would undergo a series of transformation, from hydrated metal cations to hydroxylated monomeric species, polymeric species, and finally to crystalline oxide precipitates after aging (Kushwaha et al., 2012). For example, Ni was found to exist mainly in the form of Ni ions (90%) at pH 1.0-7.0, but 10% of $\text{Ni}_4(\text{OH})_4^{4+}$ and 8.6% of $\text{Ni}(\text{OH})^+$ were formed at pH 9.0. Other specific metal may change more drastically in response to pH. For instance, $\text{Cu}(\text{OH})_2$ precipitate was the major form of Cu(II) at pH above 6.6. Ahmad et al., (2010) found high concentration of Cd, Pb and Zn on dried activated sludge at pH 3.5-4.5 due to the precipitation in the form of $\text{Cd}(\text{OH})_2$, $\text{Pb}(\text{OH})_2$ and $\text{Zn}(\text{OH})_2$, respectively. On the other side at pH 7.5, Pb tends to precipitate as hydroxide even on activated sludge for very low total metal concentration. Several solubility products for solid $\text{Pb}(\text{OH})_2$ were found from the literature with observed initial precipitation for Pb concentration ranging from 0.1 to 1mM (Pagnanelli et al., 2009). On the other hand, Ahmad et al., (2010b) noted that Cu^{2+} and $\text{Cu}(\text{OH})^+$ are the dominant species involved in the Cu adsorption on modified activated sludge below pH 5.0, thus other species $\text{Cu}(\text{OH})_2$ and $\text{Cu}(\text{OH})_3^{-1}$ were not accounted in the formation of surface precipitation. Therefore, it could be indicated that pH kept the important role for Cu adsorption by hydroxyl precipitate creation. Pontoni et al., (2016a) established the speciation diagrams to indicate the effect of pH on different Cu concentration (Figure 10). They found that at pH 6.5 there are none – negligible concentration related to Cu hydroxide



precipitation. Normally, Cu precipitate significantly at pH higher than 8, and different pH and Cu concentration can create other type of Cu hydroxyl precipitation.

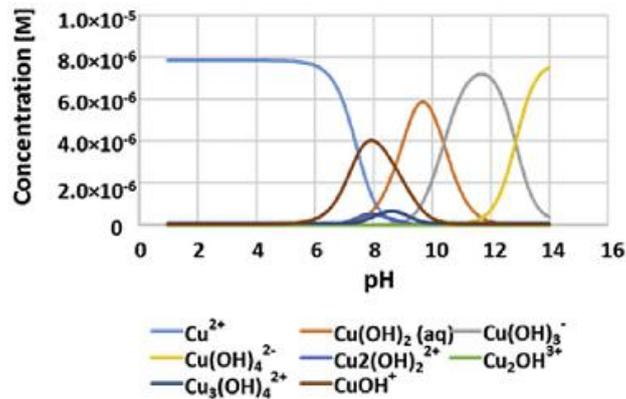


Figure 10: Speciation diagram as function of pH for Cu (Pontoni et al, 2016)

In addition, the metal ions are in competition with the H^+ in the solution at low pH values for the biosorption on biomass surface active sites. Also less functional group is ionized (deprotonated) in this region, and it is difficult that they form Cu precipitation. The study of Hg adsorption on activated sludge of Natarajan and Manivasagan, (2016) indicated that the Hg is present mainly as $\text{Hg}(\text{OH})^+$, and $\text{Hg}(\text{OH})_2$ at pH 6.0 and $\text{Hg}(\text{OH})_2$ at pH 8.0-10.0. The increase in Hg sorption efficiency in this range was due to the retention of $\text{Hg}(\text{OH})_2$ precipitation by the selected sorbent (Ghodbane and Hamdaoui, 2008). No major change in sorption efficiency was observed when $\text{pH} > 7.0$, which may be due to the precipitation of metal hydroxide (Rajamohan et al., 2014).

In case of granular sludge, it has been reported that the biosorption of heavy metal by the aerobic granules increased with the pH increasing from pH 5.0 to 7.5 because of precipitation of heavy metal under the alkaline condition (Liu et al., 2003b; Yao et al., 2009). In addition, by comparing with activated sludge, granular sludge often has higher surface capacity for adsorption of HMs due to larger functional groups distributed on surface granular sludge (Cai et al., 2014). According to the report of Xu and Liu., (2008), the mechanisms of heavy metals biosorption by the aerobic granular sludge could be attributed to the surface precipitation and intracellular adsorption. Surface precipitation refers to the aerobic granular sludge microbe's extracellular polymerize with groups like $-\text{OH}$, PO_4^{3-} and $-\text{HS}$, which carried out adsorption with metal ions by chemical precipitation reactions. Besides, Bartacek et al., (2008) indicated that the accumulation of HMs on aerobic granular sludge can be caused by or adsorption or absorption on/in the granules or by Co precipitation with inorganic ions contained in the liquid medium. The presence of carbonates (CO_3^{2-}) and phosphates (PO_4^{3-}) functional groups on granules caused CoCO_3 and $\text{Co}_3(\text{PO}_4)_2$ precipitation.

In case of anaerobic granulation under anaerobic conditions, sulfate components were reduced to sulfides that mediates the metal removal (especially Cu, Pb, Zn, Cd, Ni and Fe) via the resulting metal sulfide precipitation (Gopi Kiran et al., 2017; Sahinkaya and Yucesoy, 2010). Zandvoort et al., (2006) also indicate that precipitation of metals, e.g., as carbonates and especially as sulfides, is important for the accumulation of metals in the anaerobic granular sludge. The HMs can be retained in the anaerobic



granular sludge along the time due to the sulfide precipitates transformed from amorphous to more crystalline forms (Jansen et al., 2005). Moreover, metals precipitates (phosphate, carbonate, sulfide, etc.) may actually take part in the sludge granulation process in anaerobic granular sludge reactor (Oleszkiewicz and Romanek, 1989; Sharma and Singh, 2001; Shen et al., 1993; Yu et al., 2000). The speciation of metal on anaerobic granular sludge contents complex adsorption mechanism such as complexation, precipitation, etc. These mechanisms presented in (Zandvoort et al., 2006).

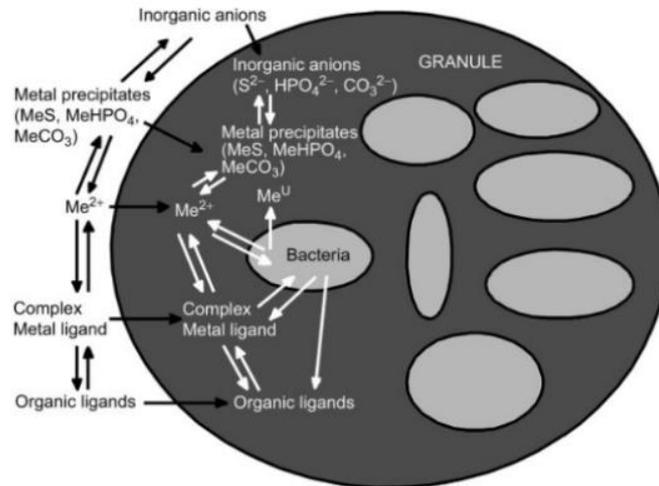


Figure 11: Metal precipitation and complexation on anaerobic granular sludge (Zandvoort et al. 2006)

EPS also is important factor during HMs adsorption on granular sludge by precipitation together with other sorption mechanism (Figure 12). There are various mechanisms involve HMs adsorption by precipitation by sludge-EPS. Surface precipitation may contribute to high metal adsorption by EPS content on sludge (Li and Yu, 2014). At neutral or alkaline pH, Cu^{2+} can easily be precipitated into EPS or onto the surface of some mineral fractions contained in EPS (Fang et al., 2011). Therefore, the chemical species of metals in solution can vary considerably with the metal category and pH, as a consequence their surface charge and absorbability may also differ significantly. These factors, along with the inherent metal absorbability of some mineral fractions in EPS, would greatly complicate the metal sorption process.

II.2.2. Biosorption capacity of heavy metal on sludge

• Definition

Biosorption of heavy metals is a relative rapid process and usually reaches equilibrium within several hours. The adsorption process experiences a fast initial sorption followed by a slower and longer uptake. At the beginning of adsorption, a large number of vacant active site are available for heavy metals, and the driving force provide by the metal concentration differences between the sludge surface and solution is large (Yunus Pamukoglu and Kargi, 2006a). As the active sites are gradually occupied by metals, the adsorption process slows down. Heavy metals can enter the pores within the sludge and subsequently get adsorbed. However, the interior adsorption needs to overcome lager mass transfer resistance (Gulnaz et al., 2006).



- **Mechanisms involved**

Bioremediation of heavy metal can occur through several pathways.

In many biosorption processes, several mechanisms take place simultaneously and it is thus difficult to distinguish the individual mechanisms (Gai et al., 2008). Table 4 summarizes the contribution of different mechanisms in some biosorption process. Among heavy metal adsorption on aerobic granular sludge, ion exchange seems to be dominant except in the study by (Yao et al., 2009), where complexation is mainly responsible for the heavy metal uptake. In case of heavy metal adsorption on anaerobic granular sludge, precipitation is dominant adsorption mechanism. Activated sludge presented different adsorption mechanisms for heavy metals. This phenomenon can be understood by different activated sludge characteristic and experimental condition for biosorption of heavy metal on activated sludge.

- **Comparison between CAS and granular sludge**

The activated sludge biomass is known as a rich organic mass and composes of microorganisms (bacteria, algae and protozoa) and inorganics. Artola et al., (1997) found that the sorption of metal by the sludge is a consequence of the interaction between metal ions in the aqueous phase and the cell wall of bacteria. The biochemical composition of this organic mass is protein, lipid, extra cellular polysaccharides, and nucleic acids. Cell wall compositions and other cellular compounds of the microorganisms are able to interact with metal ions in aqueous solutions (Gulnaz et al., 2006). The adsorption of metal ions through biomass cells and the leaching of heavy metal ions in the activated sludge biomass system seemed to be influenced by all passive interaction of the microbial cell walls with metal ions (in this case, heavy metals adsorb to carboxylic, hydroxyl, phosphate, carbonate and sulfonate groups of the lipids, proteins and polysaccharides found on the cell surfaces) (Roane et al., 2001). Summary of HMs biosorption involves the following many mechanisms such as ion exchange; physical adsorption; precipitation; complexation, etc., on sludge as biosorbent was presented in Figure 12 and Table 4.

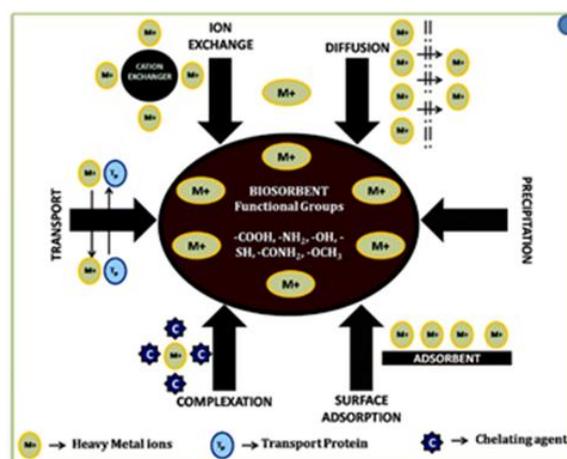


Figure 12: HMs adsorption mechanisms on conventional sludge as biosorbent (Silveira et al., 2003a).



Table 4: Contribution of different mechanisms to heavy metals biosorption on different kind of sludge: conventional activated sludge and granular sludge.

Adsorbent	Metal ion	Adsorption mechanisms			Refs
		Ion exchange (%)	Complexation (%)	Precipitation (%)	
Activated sludge	Cd (II)	57	-	0	Remenárová et al.,(2012)
	Zn (II)	66	-	0	
	Cd (II)	-	-	20	Laurent et al., (2010)
	Cu (II)	-	-	49	
Cu (II)	10	70	18	Cai et al., (2007)	
Zn (II)	39	21	10		
Aerobic granules	Cd (II)	75.51	19.36	5.31	Xu and Liu, (2008a)
	Cu (II)	71.73	16.19	12.50	
	Ni (II)	82.43	14.20	3.37	
	Pb (II)	51	20	29	Hawari and Mulligan, (2006b)
	Cu (II)	77	18	0	
	Cd (II)	82	15	0	
	Ni (II)	98	0	0	
Cr (III)	11.2	60.3	18.7	Yao et al., (2009)	
Cu (II)	70	-	-	Gai et al., (2008)	
Anaerobic granular sludge	Co (II)	-	-	66.7	Zhou et al.,(2013a)
	Ni (II)	-	-	>99	Bartacek et al., (2010a)
	Cu (II)	6.28	2.34	51.6	Zhang et al., (2015a)

Aerobic granules played a promising role in biosorption of heavy metals that may be attributed to following reasons (Wei et al., 2016):

- Positively charged heavy metals and negatively charged cell walls occur commonly in sorption process.
- Cell walls or EPS containing abundant functional groups provide rich sorption sites for heavy metal sorption.
- Ion exchange process with light metal ions (Ca^{2+} , K^+ , Na^+).
- Chemical precipitation.

Bartacek et al., (2010b) studied the mechanism of Ni sorption onto anaerobic granular sludge. They indicated the sorption process consists of three distinctive periods:



- A fast initial sorption in the first minutes upon nickel injection;
- A slow sorption process limited by intra-particle diffusion and
- Final equilibrium establishment.

Zhang et al., (2015a) studied the fate of Cu adsorption on anammox granular sludge (Figure 13). They indicated that Cu migrated from the soluble EPS (S-EPS) to the bound EPS (B-EPS) and then accumulated on the cell surfaces, entered the cells or converted to inert cores. This migration behavior may be due to a multiple – layer structure with two distinct regions in the anammox granules.

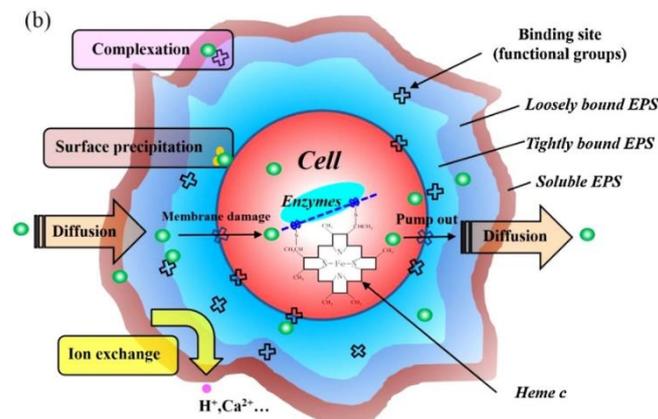


Figure 13: Behavior of Cu adsorption on anammox granular sludge (Zhang et al., 2015b).

II.2.3. Modeling adsorption ability

During the study of the retention of a soluble compound on a solid phase, it is possible to comparing the solution concentration C_e (mg/L or mol/L) and concentration retained on the solid Q (mg/g or mol/kg). The relation $Q = f(C)$ is called “sorption isotherm”. For this relationship is valued, two conditions must be satisfied: the balance physicochemical retention/release to be achieved and all other physio-chemical parameter (pH, temperature, etc.) must be constant.

The isotherms were classified into four types in Figure 14.



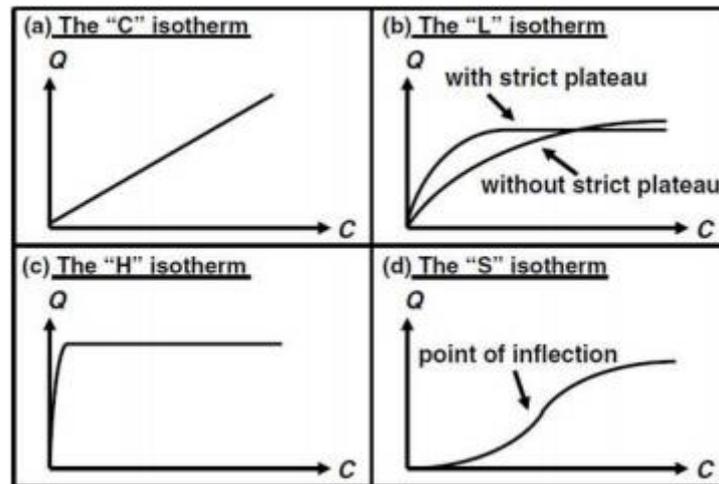


Figure 14: Representation of the four main type of isothermal (Limousin et al., 2007).

The isotherm ‘C’: the curve is a straight line passing through the origin. This means that the C/Q is always the same regardless of the concentration. This ratio is usually called “distribution coefficient” or “partition coefficient” and is expressed in L/kg.

The isotherm ‘L’: the ratio C/Q decrease as the concentration of the solute increase giving a concave curve. This suggests a gradual situation of the solid. Two subgroups can be distinguished: either the curve reaches a strict tray (the solid in a limited sorption capacity) or no plateau is observed.

The isotherm ‘H’: this is special case of the isotherm L. In that case initial slope of a vertical “infinite slop”, although this is not a strictly thermodynamic standpoint.

The isotherm ‘S’: Curve is sigmoidal shape and thus has a point inflection. This type of isotherm is met in two cases: in one hand, where a compound is more easily adsorbed on a surface already covered by the soluble, and on the other hand, the presence of a soluble ligand which will limit the sorption phenomena. The meal species may be subject to this second phenomenon: low metal concentration, sorption is limited by the presence of sorption takes place. The inflection point then illustrates the concentration at which the sorption exceeds complexation solution.

The most commonly used for modeling their models are those of Freundlich and Langmuir (Limousin et al., 2007).

II.2.3.1 Freundlich model

Freundlich isotherm is an empirical equation. This equation is one among the most widely used isotherm is capable of describing the adsorption of organic and inorganic compounds on a wide variety of adsorbents including biosorbent. This equation has the following form.

Equation 1 $q_e = K_f C_e^{1/n}$



Equation 1 can also be expressed in the linearized logarithmic form:

$$\text{Equation 2} \quad \log q_e = \log K_f + \frac{1}{n} \log C_e$$

Where q_e represents the metal phase concentration after equilibrium (mg/g), C_e is the final metal concentration in the solution at equilibrium (mg/L) of adsorbed in solution; K_f and n are Freundlich constant incorporating the factors affecting the adsorption capacity and adsorption intensity respectively. The plot of $\log q_e$ against $\log C_e$ gives a liner graph slope $1/n$ and intercepts $\log K_f$ from which n and K_f can be calculated respectively.

On average, a favorable adsorption tends to have Freundlich constant n between 1 and 10. Larger value of n (smaller value of $1/n$) implies stronger interaction between biosorbent and heavy metal while $1/n$ equal to 1 indicates linear adsorption leading to identical adsorption energies for all sites. When n value of higher than unity (n less than one), it suggests the presence of curved upward isotherm, sometime called as solvent – affinity type isotherm. Within this type of isotherm, the marginal sorption energy increases with increasing surface concentration. In this case, strong adsorption of solvent as a result of strong intermolecular attraction within the adsorbent layers occurs (Site, 2001).

Adsorption capacity is the most important characteristic of an adsorbent. It is defined as the amount of adsorbate taken up by the adsorbent per unit mass of adsorbent. This variable is governed by a series of properties, such as pore and particle size distribution, specific surface area, cation exchange capacity, pH, surface functional groups, and also temperature. Most of the adsorption capacity for biosorbent (obtained from Freundlich K_f parameter) is quite low. Different types of bio sorbents are still attractive due to its biosorption advantages and cost-effectiveness for metal biosorption (Febrianto et al., 2009).

As a robust equation, Freundlich isotherm has the ability to fit nearly all experimental adsorption-desorption data, and is especially excellent for fitting data from highly heterogeneous sorbent system. Accordingly, this isotherm can adequately represent the biosorption isotherm for most of the systems studied. Still, in some cases, Freundlich isotherm could not fit the experiment data well (as pointed by the low correlation values) or not even suitable for the biosorption equilibrium expression (Baral et al., 2007).

As a precaution note, Freundlich equation is unable to predict adsorption equilibria data at extreme concentration. Furthermore, this equation is not reduced to linear adsorption expression at very low concentration. Moreover, it does not have limit expression at very high concentration. The concentration of biosorbents needs to be ranged before experiments.

II.2.3.2 Langmuir model

Langmuir model is widespread – used model for describing heavy metals sorption to sludge as biosorbent. Langmuir equation relates the coverage of molecules on a solid surface to concentration of a medium above the solid surface at a fixed experiment condition of temperature. This isotherm based on the assumption, namely adsorption is limited to monolayer coverage, all surface sites are alike and only can accommodate one adsorption atom and the ability of molecules to be adsorbed on a given site is independent of its neighboring sites occupancy. By applying these assumptions, and a kinetic



principle (rate of adsorption and desorption from the surface is equal). The Langmuir equation can be written in the following form:

$$\text{Equation 3} \quad q_e = q_{\max} \frac{K_L C_e}{1 + K_L C_e}$$

This equation is often written in the linear forms:

$$\text{Equation 4} \quad \frac{C_e}{q_e} = \frac{1}{q_{\max}} C_e + \frac{1}{K_L q_{\max}}$$

Where: q_e is amount of compound sorbed (g/kg or mol/kg) and C_e is concentration of compound in soluble (g/L or mol/L) at equilibrium; q_{\max} is maximum adsorption capacity of the solid studied (g/kg or mol/kg), K_L : Langmuir affinity constant (L/g or g/mol).

Langmuir model assumes uniform energies of sorption onto a homogenous sorbent surface and no migration of the sorbate in the plane of the surface. Because sludge particulates are the heterogeneous materials, the information derived from the application of the Langmuir model must be taken with precaution. However, and as pointed out for the Freundlich model, it is a model also widely applied to describe metal sorption in sludge or soil (Febrianto et al., 2009). Base on analysis parameters, we can have more information of the adsorption processes such as the metal binding takes place as a passive mechanism based on the chemical properties of surface functional groups (Hammami et al., 2007a). The mechanism involved in metal adsorption are complicated, therefore the interpretation is very difficult (Zorpas et al., 2011). Usually these mechanisms are related to electrostatic interaction, surface complexation, ion-exchange, and precipitation, which can occur individually or in combination. Moreover, pretreatment of adsorbents improves physical and chemical properties of bio-sorbent, increase the adsorption capacity and prevents organic leaching, while chemical modification makes some improvements on surface active sites, liberates new adsorption sites and enhances mechanical stability and protonation (Laurent et al., 2011a).

- **Comparison between CAS and granular sludge**

Conventional activated sludge and granular sludge show limited differences in metal uptake capacity in batch mode studies. The maximum adsorption capacity as main factor indicates the heavy metal biosorption capacity on the sludge. Table 5 summarizes the maximum adsorption capacities of sludge. As can be seen from Table 5 granular sludge has comparable adsorption capacities with activated sludge. Furthermore, the dense structure and excellent settling ability of granular sludge make granular sludge feasible for heavy metals sorption.

Adsorption capacity is highly dependent, for a considered metal, on the kind of sludge (CAS/granular sludge), and on the kind of implementation process. In the same sludge treatment method such as dried CAS, almost studied shows higher Cu adsorption ability in sludge created from real industrial wastewater than that in domestic synthetic wastewater. Besides, for instance, with the same type of domestic wastewater, granular sludge can enhanced more Cu adsorption capacity, compared to CAS. This could be explained base on denser structures of granular sludge and diverse characteristics of organic functional groups which lead Cu easier for adsorption.



Table 5: Maximum adsorption capacities (q_{max}) of different sludge towards heavy metals

Sludge	Sources of sludge	Heavy metals	Adsorption capacity (mg/g)	References
Powdered activated sludge	WWTP (Paint company)	Cu(II)	27.7	Kargi and Cikla, 2006; Pamukoglu and Kargi, (2007)
		Zn (II)	82.2	
Dried activated sludge	WWTP (Paper company)	Cu(II)	91	Zare et al., (2015)
	SBR (lab scale reactor)	Cu (II)	10.6	Ong et al., (2013)
		Cd (II)	9.6	
		Ni (II)	18.6	
Cu (II)	20	Lawson et al., (1984)		
Cd (II)	13			
Ni (II)	11			
Zn (II)	3			
Cr (III)	10			
Aerobic granules	SBR (lab scale reactor)	Zn (II)	64.44	Wei et al., (2016)
Disintegrated aerobic granules	SBR (lab scale reactor)	Cu(II)	40.71	Wang et al., (2010a)
Aerobic granular sludge	SBR (lab scale reactor)	Sr (II)	28.8	Li Wang et al., (2013)
		Cu (II)	121.79	Luo et al., (2016)
		Cd (II)	566	Liu et al., (2003c)
		Ni (II)	48.27	Xu et al., (2006a)
Anaerobic granular sludge	UASB full-scale reactor – (paper mill wastewater)	Co (III)	18.76	van Hullebusch et al., (2006)
	UASB full-scale reactor – (alcohol wastewater)	Ni (II)	6.5	Bartacek et al., (2010c)
	UASB reactor – (cheese production wasteater)	Pb (II)	255	Hawari and Mulligan, (2006c)
	Cd (II)	60		
Cu(II)	55			
Ni(II)	26			
UASB full-scale reactor- (paper mill wastewater)	Co (III)	11.71	Osuna et al., (2004)	
Ni (II)	13.33			



II.2.4. Factors affecting biosorption

Biosorption is highly dependent on technology of wastewater treatment, type of concentration and oxidation state of metal, sludge and influent composition, pH of wastewater, type of microorganism (Bartacek et al., 2010c; Zhang et al., 2015b). So, study on effecting factors on adsorption is the key to understand the heavy metal bioavailability on sludge.

II.2.4.1 Element type properties

The sorption of heavy metals depends on the type, properties and nature of the elements involved (Ceçen et al., 2010; Klapwijk et al., 1974; Ong et al., 2005a).

- **Comparison between CAS and granular sludge**

Among the numerous methods to measure metal adsorption capacity (Ceçen et al., 2010; Klapwijk et al., 1974; Ong et al., 2005a). It has been difficult to compare results obtained with the different methods. For example, the following sequences of heavy metal adsorption on bacterial communities in activated sludge have been reported: $Cd > Cu > Zn > Cr > Pb$, $Cd > Cr > Cu > Zn$. These discrepancies between the two samples probably resulted from both the type and structure of the bacterial which affected the adsorption of heavy metal on sludge.

Generally, increasing the heavy metal concentrations in sludge resulted in a corresponding the adsorption of heavy metal. The study of (Lawson et al., 1984) showed that Cu is the most adsorbed on activated sludge microorganisms. Other study of Ong et al., (2005b) indicated Cd is less adsorbed than Ni. The several of Ni and Cd in the adsorption could probably be explained by the reduction of these metals during oxygen uptake of the sludge microorganisms. Heavy metal adsorption experiments performed on activated sludge reveal an uptake sequence of $Cu > Ni > Cd = Cr > Zn$ (Lawson et al., 1984).

The relationship between anaerobic granular sludge and heavy metals adsorption were directly proportional to metal properties. It was reported that the adsorption of Zn and Pb on aerobic granular sludge as greater than Cd at the same pH and experimental conditions (Ahmad et al., 2010a). By comparison between Co and Zn adsorption, aerobic granular sludge can adsorb Co alone more rapidly than Zn alone from aqueous solutions (Sun et al., 2008). In the sense of chemical reactions, the reaction order is directly related to the reaction mechanisms.

II.2.4.2 Heavy metal concentrations

High heavy metal adsorption capacity depends on sorption sites of high selectivity, which have relatively strong bonding energies. Otherwise, heavy metal sorption becomes unspecific at higher element concentrations, when the specific bonding sites become increasing occupied (Pamukoglu and Kargi, 2007).

At low element concentrations, elements are mainly adsorbed onto specific sorption sites, while at higher element concentrations, sludge lose some of their ability to binding trace elements as sorption sites overlap, thus becoming less specific for particular metal ions. This in turn, induces a reduction in element sorption (Yunus Pamukoglu and Kargi, 2007). The sorption isotherms, which were best described



by the Freundlich curve fit model (L-curve type), shows relatively high affinity of sludge for adsorbates at low element concentrations. This affinity decreases with an increase in the proportion of the surface covered with the adsorbate (Ahmad et al., 2010a). It is possible to distinguish two types of sorption sites:

- High affinity sites, which responsible for element sorption at low added element concentration
 - Low affinity sites, which are responsible for element retention at higher added element concentrations
- **Comparison between CAS and granular sludge**

Pamukoglu and Kargi, (2007) study the Cu adsorption on conventional activated sludge in the range of Cu ions concentration between 50 and 300 mg/L. They found that at low Cu ion concentration the binding sites on the sludge surfaces were in excess of Cu ion available in aqueous phase yielding low Cu ions concentration in solution. However, when the feed Cu ion was increased, Cu ion concentration exceeded the binding sites on sludge surfaces yielding high Cu ion concentration in solution (Pamukoglu and Kargi, 2007).

Initial metal concentration provides an important driving force to overcome all mass transfer resistances of the metal ion between the aqueous and solid phases. Hence, a higher initial concentration of heavy metal ions will enhance the biosorption process (Gulnaz et al., 2006). The study of Benaïssa and Elouchdi (2011) obtained the amount of Cu biosorbed on drying activated sludge at the equilibrium increases with the initial Cu concentration: 9.21 mg/g ($C_0 = 20$ mg/L) and 59.41 mg/g ($C_0 = 500$ mg/L).

Zhou et al., (2013) study the adsorption of Cu on fresh powdered anaerobic granular sludge (PAGS) and disintegrated anaerobic granular sludge (DSR- PAGS) when the initial concentration of Cu (II) changed from 100 mg/L to 400 mg/L. At initial Cu concentration of 100 mg/L, the adsorption amount of copper by methanogenic PAGS and DSR-PAGS was only 17.65 and 8.71 mgCu/g PAGS. When the initial concentration of Cu (II) increased to 400 mg/L, the adsorption amount of copper by methanogenic PAGS and DSR-PAGS were increased to 39.01 and 27.94 mgCu/g PAGS, respectively. Higher initial concentration could improve the transfer of metal ions from solution to solid phase.

II.2.4.3 Sludge properties

- **EPS composition and chemical functions associated**

The metal ions can be retained by some of the microbial biomass through the interaction between the positively charged metal ions and the binding sites on the cellular wall of microbial biomass.

In general, almost heavy metal adsorption on sludge studies focused on the maximum adsorption capacity of a certain kind of waste sludge (Table 5). These studies failed to investigated the source and characteristics of the sludge used for the biosorption process. In fact, sludge samples have often very different physico-chemical properties due to the kind of process implemented and to the composition of the effluent (Lister and Line, 2001). Therefore, the EPS component distribution in sludge also varied. Type of EPS functional groups will decides the main interaction between sludge and metal ion, which is individual, or complexes, complexation, ion exchange and/or precipitation, etc, (Figure 15)



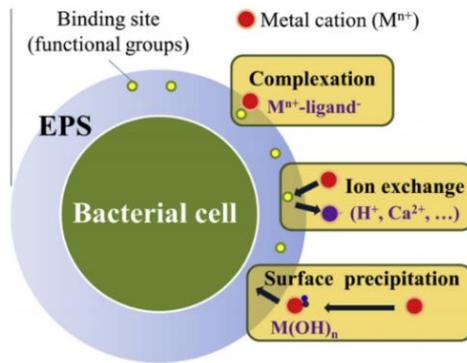


Figure 15: Schematic diagram of mechanism of metal - EPS interaction and precipitation (Li and Yu, 2014).

In the last decades, a lot of publications demonstrated that EPS composition had very strong influence on trace metal element sorption ability in waste activated sludge (Guibaud et al., 2009). In addition, thermal hydrolysis of activated sludge can increase the binding site on surface, therefore enhance HMs adsorption by complexation and precipitation with functional groups such as carboxyl or phosphate on disintegration of activated sludge (Laurent et al., 2011b). Hence, activated sludge could be considered as a biosorbent with different functional groups such as carboxylic acid, carboxyl and amine groups (Gulnaz et al., 2006; Laurent et al., 2011a).

Several study aimed at explaining the functional groups and adsorption mechanisms between heavy metals and biopolymer. Yuan et al., (2011) revealed that carboxyl and hydroxyl groups have been found to be primarily involved in the heavy metal complexation by forming stable complexes under neutral pH solution. Phosphoric amine and amidocyanogen of proteins, polysaccharides and phospholipids could also become negatively charged and serve as effective metal binding ligands. Ozdemir et al.,(2003) also found that the carboxyl groups were negatively charged in neutral pH solution, and attracted positively charged cations through electrostatic interaction and form organometallic complex. Fang et al., (2011) further mentioned that nitrogen in amino-sugars and oxygen in hydroxyl and carboxyl were the main electron donor atoms, which were prone to preferentially bind with soft metal cations of strong covalent characteristics and then form inner – sphere complexes.

Moreover, the formation of functional groups in sludge could be enhanced with pretreatment method such as heating, autoclaving, acidic treatment and alkali treatment and then improve trace metal element sorption (Kargi and Cikla, 2006; Shroff and Vaidya, 2012).

Zare et al., (2015) compared the adsorption capacities of Cu, Ni, and Cd ions onto the fresh activated sludge and dried sludge (at 105°C and 24h). They indicated that the adsorption capacity of these ions on fresh activated sludge were significantly greater than dried sludge according to the kinetic models.

Many studies have shown that aldehyde, carboxylic, sulfhydryl, phosphoryl, hydroxyls, amine organic and acidic functional groups of sludge were implicated metal ions removal from aqueous solutions (Zhang and Banks, 2006). Kargi and Cikla (2006) demonstrated that waste activated sludge, pre-treated with 1% H₂O₂ improve the Cu biosorption capacity. The author hypothesis that H₂O₂ oxidized or



activated some functional groups on the surfaces of bacterial biomass and provided more suitable surface characteristics for attachment of Cu ions (Pamukoglu and Kargi, 2006).

The ability of dried anaerobic activated sludge to adsorb phenol and chromium (VI) ions in a batch system were investigated by (Aksu et al., 2002). The cells of dried anaerobic activated sludge bacteria were effective for simultaneous removal and separation of phenol and Cr (VI) ions from aqueous effluents.

- **Case of granular sludge**

Among different kinds of waste sludge, anaerobic granular sludge often has higher biomass content. Obviously, characteristic of such waste sludge might depend on the treatment processes in many ways and affect the heavy metal adsorption. Anaerobic granular characteristics such as growth environment, microbial community and physical structure affected largely the biosorption capacity.

However, the mechanisms of binding between the material and the EPS molecules are not well understood and a lot of studies on the mechanisms of the binding of heavy metals to sludge conclude that different binding sites are involved depending on the type of sludge (cell wall, EPS, polysaccharides, etc.) and the experimental conditions (pH, temperature, ionic strength, metal ions, concentration, ratio bacterial mats/metal ions, etc.). Table 6 presented the effect of EPS on heavy metal adsorption on sludge. EPS components of sludge were extracted by different method according to the different studies.

Liu et al., (2003d) studied the feasibility of aerobic granules for Cd removal from industrial wastewater. A kinetic model was developed to describe Cd biosorption by aerobic granules. Results showed that the Cd biosorption on aerobic surface was closely related to both initial Cd and granule concentrations.

Guibaud et al., (2012a) investigated Cd (II) and Pb (II) sorption by EPS extracted from anaerobic granular biofilms. EPS displayed a better binding ability for Pb (II) than Cd (II) due to the strong binding capacity between heavy metal and EPS. Heavy metal may be trapped in the network of EPS matrix to form metal-EPS-complex during sorption process.

For granular sludge, EPS plays the primary role in the adsorption of heavy metal ions on granules. According to the research of Liu et al., (2015a), the abundant carboxyl hydroxyl groups located on protein are the main adsorption sites, and the high protein content of EPS leads directly to the extremely large capacity for adsorption of heavy metal ions. Moreover, the metal ions (such as Pb, Cd, and Zn) are adsorbed onto EPS through the complexation function by means of $-\text{COO}-$ and $-\text{OH}$ under laboratory conditions. Figure 16 presented a close relation between the good adsorption properties of granules, the excellent adsorption performance of EPS and the high protein content of EPS (Liu et al., 2015b).



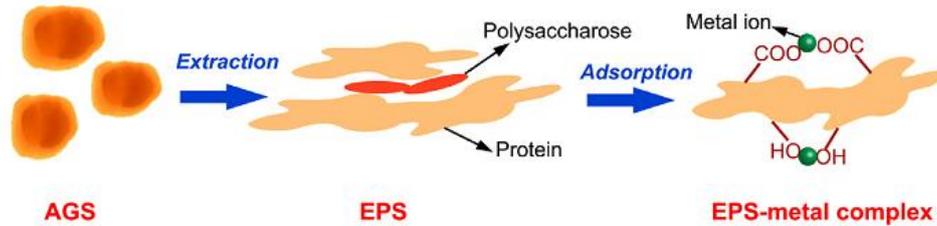


Figure 16: Schematic diagram of adsorption of heavy metal ions onto EPS extracted from aerobic granular sludge (Liu et al., 2015b).

According to Table 6, the removal of metal ions via either activated sludge system or granular process can be enhanced by improving the PN content in the EPS as biopolymer. During activated/granular sludge formation, nutrient (C, N, COD., etc.) levels and proportion in wastewater have significant effect on the composition of EPS (Durmaz and Sanin, 2001). As for the EPS extracted as adsorbent, the extraction method of the EPS can be adjusted to obtain high PN and/or PS proportion which depend on sludge characteristic and purposes of study. Almost results suggested CER was the best and popular method to extracted high protein content biopolymers from activated/granular sludge.



Table 6: The effect of EPS components on sludge to heavy metal adsorption

Sludge	Heavy metals	EPS extraction methods	EPS components	Role of EPS on adsorption process	Refs
Activated sludge	Cu (II)	Phosphate buffer solution and CER	PN: 25.49mg/gVSS PS: 139.30 mg/gVSS	EPS-PN as biosorbent of Cu was 2.34 times as much as the adsorption capacity of disintegrated aerobic granules.	Wang et al., (2010a)
	Cu (II)	Ultrasound reactor (20kHz)	PN: 52.6% PS: 30.7% NA: 16.7%	The carboxylic groups and amino groups may coordinate to Cd and Zn ions as chelating (bidentate) complexes.	Z. Zhang et al.,(2014a)
	Cu(II)	Series of bench scale of chemical compositions	PN: 21% - 35% PS: 24 - 70% NA: 9 - 41%	PN were identified to be the key constituents and feature of the biopolymer responsible for binding heavy metals.	Zhou et al., (2016b)
Aerobic granules	Zn(II)	The heat method	PS: 5.92 mg/g PN: 23.55 mg/g	PN-like substances were more susceptible to Zn (II) binding than humic – like substances. The main functional groups for complexation of Zn and EPS were O-H and C=O groups.	Wei et al., (2016)
	Pb (II) Cd (II) Zn (II)	CER (75gCER/gVSS)	PN: 86.8% PS: 9.7% HS: 2.3%	Dominant adsorption mechanisms were complexation by carboxyl and hydroxyl groups of PN-heavy metals.	Liu et al., (2015c)
	Pb (II) Cd (II)	Nine different techniques	The values varied according to the extraction methods	Pb and Cd seem to bound different with the EPS, a higher binding capacity was observed for Pb than for Cd and extraction protocol has strong effect to adsorption process.	d'Abzac et al., (2010)
Anaerobic granular sludge	Cu(II)	The heat extraction method	Not mention	The cu migrated from the soluble EPS (S-EPS) to the bound (B-EPS) and then accumulated on the cell surfaces, entered the cell or converted to inert cores during exposure to the over loaded environment.	Zhang et al., (2015a)
	Pb(II) Cd(II)	CER (70g CER/gVSS)	PN: 91.8 mg/g; HS: 120.6 mg/g PS: 93.1 mg/g; UA: 4.4 mg/g NA: 7.0 mg/g;	Mechanism of proton exchange is likely to be involved in the Cd or Pb binding by EPS extracted from anaerobic granular sludge. Pb displays a better affinity for EPS than Cd as function of pH.	Guibaud et al., (2012b)

PS : Protein components ; *PS* : Polysaccharides component ; *HS* : Humic like substances ; *NA* : Nucleic acids components ; *UA* : Uronic acid components



As previously observed for waste activated sludge, granular sludge treatments could impact their sorption ability.

- **Thermal treatment**

Zhou et al., (2011) studied the biosorption efficiency of Cu ion on methanogenic anaerobic granules and “thermally dried” anaerobic granules at three different temperatures: 25°C; 35°C and 45°C. They found that the biosorption efficiency of Cu ion on fresh methanogenic anaerobic granules was always higher than dried anaerobic granules. By comparing adsorption at 25°C to that at 45°C, the biosorption amount of Cu²⁺ by fresh anaerobic granules decreased by 68%, while dried anaerobic granules decreased by 60%.

Hawari and Mulligan, (2006c) studied the biosorption of Pb, Cd, Cu and Ni by viable /non-viable anaerobic granular biomass. A non-viable anaerobic granule was prepared by during anaerobic biomass at 50°C for 6 days. Three viable biomass obtained separately from nonviable biomass was treated with 0.02 M HCl, 0.02 M KOH and 0.01 M Ca(OH)₂. They found that for the 4 metal ions studied, the uptake capacity of the viable biomass was higher than that of the nonviable biomass. For the viable biomass the metal uptake is also facilitated by the production of metal-binding proteins.

Therefore, the higher uptake capacity of the viable biomass could be due to the active uptake of the biomass. Active uptake by the cell membrane usually requires energy from the cell for the transfer process. This process can be highly selective and often irreversible unless the living system is destroyed.

Another possible explanation why sorption was higher for the viable biomass is the difference of surface area of viable and non-viable biomass. The biomass was killed by basically drying the biomass in the oven. Water was most abundant single compounds in the cell and it makes almost 70% of the total weight of the cell. Thus by drying the biomass into the form of pellets the surface area of the cell would decrease, i.e. less area is exposed to the metal ions, therefore, less metal uptake.

Furthermore, according to the analysis of specific surface area, the dried powdered methanogenic granules (6.7957 m²/g) had much more surface area than the powdered disintegrated granules (3.7889 m²/g). Obviously, the methanogenic anaerobic granules, which had less smooth structure and more surface area, could have a better biosorption capacity.

- **Disintegration**

The study of Wang et al., (2010) aimed to investigate the performance and mechanisms of the adsorption of heavy metals by disintegrated aerobic granular sludge. Cu was selected as a typical candidate of heavy metals pollutions. Disintegrated aerobic granular sludge holds greater potential for the removal of Cu than initial aerobic granules, and Cu biosorption capacity increased with the increasing of pH (3 – 5). Cu biosorption was associated with a significant release of Ca from disintegrated aerobic granules. EPS played an important role in biosorption, but its efficiency was restricted by the spatial structure of



granules. Functional groups of disintegrated aerobic granules contributed to Cu adsorption in the order of $-\text{COOH} > -\text{NH}_2 = -\text{OH} > -\text{PO}_4^{3-}$.

Hence, sludge characteristics have strong effects to heavy metals adsorption. In our knowledge, very limit research study on comparing of heavy metal adsorption capacity on activated sludge and granular sludge. But from these points of view we can see that the different sludge properties between activated sludge and granular sludge might affect to heavy metal adsorption capacity. These comparisons need to be obtained from the same experimental conditions such as sludge environmental creation, heavy metals species, sludge treatment method, etc.

- **Granules**

Adsorption is a mass transfer that depends on effectively surface contact between the target metal ions with the solid sludge surface. Generally, mixing condition in adsorption reactor provide better contact between the heavy metal and sludge since heavy metals are not in contact with each other due to complete mixing. Mixing can improve the rate of adsorption due to complete interaction between the phases, which provides a homogeneous mixture and therefore better control of the environmental conditions.

At high metal ions and low sludge concentration the rate is limited by the availability of sludge surface or binding sites. On the contrary, a limitation of adsorption was observed at high metal concentration and large particles. Accordingly, the adsorption rate increases with decreasing particle size. The total surface area of sludge particles increases at small particle size, thus leading to an improvement of the heavy metal binding to functional group on the surface of sludge and to a rise of adsorption efficiencies (Yunus Pamukoglu and Kargi, 2007).

- **Comparison surface impact to HMs adsorption between CAS and granular sludge**

As mention above, various types of sludge have been used as biopolymer for binding heavy metals, such as sewage activated sludge (Al-Qodah, 2006; Hammami et al., 2007b), dried activated sludge (Aksu et al., 2002; Wang et al., 2006), aerobic digested activated sludge (Z. Zhang et al., 2014b; Zhou et al., 2016a), alkali modified sewage sludge (Al-Qodah, 2006) and aerobic granular sludge (Liu et al., 2003c). Macromolecular biopolymers in the surface sludge play an important role in the adsorption of heavy metals by sludge (G.-P. Sheng et al., 2010b). Aerobic granules often produced by self – aggregation of suspended flocs activated sludge have more compact and stronger structure and larger surface area than activated flocs structure (Ozdemir et al., 2003b). Compared with the sludge flocs, the maximum HMs adsorption amount for aerobic granules was much higher (Gai et al., 2008; Xu and Liu, 2008b), which could be attributed to their compact structure, greater biomass preservation and high surface area (Gonzalez-Gil and Holliger, 2014; Liu et al., 2009b). They also demonstrated that surface complexation might have occurred between hydroxyl group and HMs.



Aerobic granules could be used as an effective adsorbent to remove Cu due to their large specific surface (Wang et al., 2010b).

Wei et al., (2017) compared the Zn, Cu adsorption ability on EPS of three kinds of sludge: activated sludge, anaerobic flocs sludge and anaerobic granular sludge. They indicated that surface polarity (hydrophobic/hydrophilic) distribution of the sludge EPS affected the bio-adsorption processes of Zn and Cu. Surface hydrophobic of sludge EPS had a relative higher specific surface area and complex macromolecular structure of anaerobic granules than that of anaerobic flocs and activated sludge which led to higher Cu and Zn adsorption on anaerobic granules. Hence, they proposed that the hydrophobic EPS combined with the element Zn and Cu preferentially and formed the EPS/heavy metal-complex on the surface anaerobic granular sludge at the initial stage of adsorption.

Ozdes et al., (2014) assumes uniform energies of sorption onto the surface and no transmigration of the sorbate consequence, the surface adsorption of HMs on sludge determination by the Langmuir model could be used. The value of maximum adsorption density Q_0 indicated that anaerobic granular sludge was efficient in surface adsorbing the heavy metal of Zn and Cu, with Q_0 value of 1157.6 mg/g for Cu and 721.7 mg/g for Zn comparing with anaerobic flocs and/or activated sludge (Wei et al., 2017).

II.2.4.4 Sludge concentration

Heavy metal ions adsorption increased at high sludge contents due to increased occupation of binding sites on biosorbent surfaces. For example, in (Pamukoglu and Kargi, 2007), Cu ion concentration decreased in the effluent with increasing amounts of the sludge due to larger surface area or binding sites on sludge surfaces at high sludge concentration.

The experiment of Pamukoglu and Kargi (2006) showed that the extent of Cu biosorption was limited by the availability of the binding sites on the sludge surface. Low biosorbent contents such as 1g or 2g resulted in high residual Cu ions in solution. At high sludge contents such as 5g or 6g of suspended solids concentration, large fractions of Cu ions were biosorbed onto sludge surfaces resulting in low residual Cu concentration in solution.

Moreover, the rate of heavy metal adsorption on sludge also increased with high sludge concentration. This is due to increasing binding sites in that condition (Yunus Pamukoglu and Kargi, 2007). However, Ahmad et al., (2010b) precise that it is necessary to take also in consideration the amount of sorbed copper as a function of sludge volume since large amounts of heavy metal ions can be removed from a lower volume of sludge solids.

The optimal sludge concentration for heavy metal adsorption depends on sludge properties and experimental conditions: at high sludge solid concentration, and increase in sludge pH, biosorption of metal ions decreased considerably (Ahmad et al., 2010a).



To date there is no comparisons of the impact of sludge concentration on HM adsorption for a given concentration of activated sludge versus granular sludge

II.2.4.5 Sludge pH

The pH in sludge solution directly and indirectly influences all chemical processes, and consequently the behavior of heavy metal adsorption on sludge. The solubility of the heavy metal that occurs as free hydrated cations generally increases with decreasing pH (Yunus Pamukoglu and Kargi, 2007). Various factors explain this behavior:

- Competition for sorption
- Decreasing pH creates more negative charge of the sorption complex
- Dissolution of sludge components

The sorption of element is generally acknowledged to be directly proportional to sludge pH. In addition, sludge pH plays a major role in the sorption by precipitation mechanisms of heavy metals due to the carbonates and phosphates ionic species. An increase in the sludge pH, increase cationic heavy metal retention to sludge surfaces via sorption, inner-sphere surface complex, and/or precipitation and multinuclear type reactions. Increased element sorption with increasing pH is attributed to changes in the hydrolysis state of ions in solution, therefore explaining heavy metal easy adsorption on sludge (Xu et al., 2006b).

As mentioned before extracellular polymeric substances (EPS) are reported to be actively involved in the biosorption of metals by sludge. In sludge, EPS form the matrix where bacterial cells are embedded and involved in the formation of microbial aggregates, adhesion to surfaces, flocculation, ions sorption and water retention (Guibaud et al., 2012b). EPS components contain many functional groups such as O-H groups, amide I, carboxyl groups, C-O-C, phosphate, etc. The interaction between heavy metal ions and these EPS functional groups depend on pH solution condition. So, EPS is important factor for heavy metal sorption on sludge.

In more details, pH is an important parameter in biosorption of metal ions since variations in pH affect the surface charge of biosorbent and also the solubility of metal ions.

- **Case of copper: conventional activated sludge.**

Yunus Pamukoglu and Kargi, (2007) found that Cu ions are known to precipitate in form of hydroxides at pH values above 5 on activated sludge. The rate of adsorption increased with increasing pH due to increasingly negative charges on sludge surfaces and lower (H^+) ion concentration competing with the Cu (II) ions at high pH values. In addition, the pH of metal bearing wastewater varies greatly (Benaïssa and Elouchdi, 2011b). The interactions between sorbate and sorbent is affected by the pH of an aqueous medium in two ways: firstly, since metal ions can have different speciation forms at different pH. Secondly, the surface of the



biosorbent consist of biopolymers with many functional groups, so the net charge on biosorbent, is also pH dependent (Villaescusa et al., 2000). The distribution diagrams (Figure 17) of Cu species as a function of pH show that only cationic species (Cu^{2+} , $\text{Cu}(\text{OH})^+$) are present at low concentration in solution, thus indicating high Cu adsorption on sludge. Accordingly, at the lower pH values, the surface charge of the biosorbent is positive, which is not favorable to Cu ion biosorption. Meanwhile, hydrogen ions compete strongly with metal ions for the active sites, resulting in less biosorption. With increasing pH from 3 to 5, electrostatic repulsions between Cu ions and surface sites and the competing effect of hydrogen ions decrease. Consequently, the metal biosorption increases. Beyond pH 5, insoluble Cu hydroxide starts precipitating resulting in lower amount of Cu biosorbed at equilibrium.

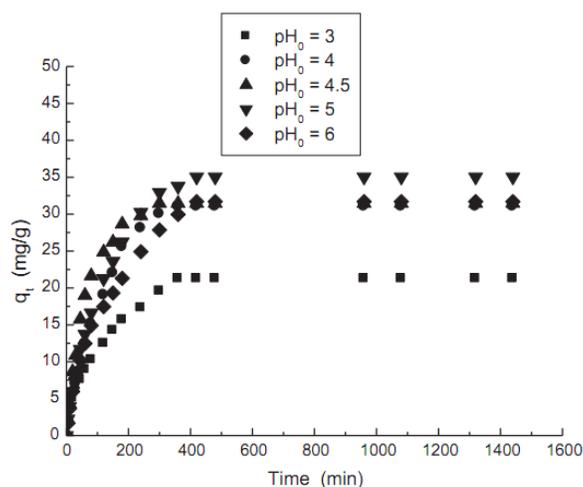


Figure 17: Effect of initial pH on the kinetics of Cu biosorption by dried activated sludge (Benaïssa and Elouchdi, 2011b).

- **Case of copper: granular sludge**

Luo et al., (2016) also indicated that increasing pH had a positive effect on the Cu (II) biosorption capacity on aerobic granular sludge. They found that aerobic granules had a higher affinity constant for Cu (II) as the pH increased. They also indicated that the functional groups such as carboxyl group might be involved in complexing with Cu (II) by analyzing FITR spectra of aerobic granules before and after Cu biosorption in pH effect condition (Figure 18). The pH influences the surface charge of these functional groups of granular sludge, which consequently affect the Cu ion uptake by aerobic granules. As the pH increased, the carboxyl group would be ionized and the surface charge of sludge would become more negative, resulting in an increase in the biosorption capacity of the sludge toward Cu.



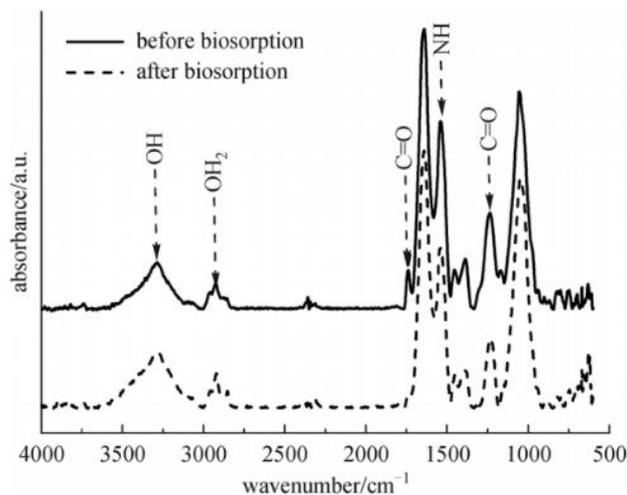


Figure 18: ATR - FTIR spectra of aerobic granules before and after Cu biosorption (Luo et al., 2016).

According to Guibaud et al., (2012a), both cations Pb and Cd are not sorbed by EPS on anaerobic granular sludge at low pH. When pH increases, the amount of Cd and Pb sorbed by EPS increases drastically. According to the relative positions of the pH – sorption, Pb appears to be more competitive toward protons than Cd on surface sites. The major part of metal of metal in solution is sorbed by EPS within a relative higher pH range, varies over pH 6. The presence of a pH sorption edge is due to 1) protons exchange between the binding sites and the metal in the bulk solution, and 2) the electrostatic binding sites (apparent pKa and number of proton binding sites) of these EPS have. Moreover, the pH range was investigated (2.6-11.5) and several functional groups harbored by EPS (i.e., carboxyl, phosphoric, amine and hydroxyl sites) are involved in Cd or Pb binding. The binding of metal ions by EPS results from the contribution of different proton binding sites, which could explain the relatively large pH range (about pH 6) of the pH-sorption.

II.2.4.6 Temperature

Temperature is reported to influence the sorption mechanism by affecting the mobility of ions and the energy of the molecules. It was widely recognized that the decrease of biosorption efficiency with elevated temperature was due to the decrease of liquid viscosity; this increased adsorbate diffusion on the adsorbent and reduced combinations between the metal ion and function groups from granular sludge (Zhou et al., 2013b).

The adsorption rate increased with increasing temperature due to high energy levels and frequency of interactions among the metal ions and the sludge particular at high temperatures. By consensus, other study also indicated that increase in temperature might reduce the thickness, viscosity and density of solution interface film, therefore promoted ions diffusion and biosorption. (L. Wang et al., 2015).



III. Heavy metals dynamic in sludge application in land spreading

III.1. Interest of sludge land spreading and legislation controlling sludge land spreading in France and in Vietnam

III.1.1. Control of sludge land spreading in France

According to data collected by the statistical regulation on waste, about 15Mt of crude product, 1.5Mt of dry matter of sewage sludge are produced in France (MEDE, 2010). This sludge is composed of water and inorganic and organic solids. Sludge after stabilization process are disposed of by burning, put in landfill or recycled in agricultural. In 2010, 639Mt of dry matter are spread on farmland as fertilizer. Today, 42% of the annual production of these urban sludge is spread on agricultural land without prior composting (about 0.4Mt DS in 2011) and 31% after composting (0.3Mt DS), 18% incinerated and 9% landfilled (EsoMafor, 2014). The spreading of industrial and sewage sludge is about 2-3% of the French agricultural area, or 285,600 ha, at 30t DS ha⁻¹ of raw material. Within 4 to 5 years usually separates two consecutive spreading of sludge on the same plot. Volunteer farmers who spread sludge on their plots are advised by the Chambers of Agriculture or private providers under state control. This practice is strictly regulated in the health and environmental point of view, to ensure both the ability of soil to perform the function of recycling and also the safety land application of the food chain and water (MEDE, 2014). In France 75% of the sludge is land spread.

Indeed, sludge may contain traces metal. To avoid trace metal enrichment of soils subjected to sludge spreading, France has adopted a regulatory framework (Decree of December 8th 1997 and Decree of January 8th 1998), going beyond the European standards (Directive 86/278/EEC) as present in Table 7, the levels of metals are analyzed in sludge and in soils prior to any application.

Table 7: Concentration limits in trace elements and trace organics for regulation of the spreading of sludge (January 8th 1998)

Heavy metals	Limit values in sludge (mg/kg DS)	Maximum amount accumulated over a duration of 10 years (g/m ²)
Cadmium	10	0.03
Chromium	1000	1.5
Copper	1000	1.5
Mercury	10	0.015
Nickel	200	0.3
Lead	800	1.5
Zinc	3000	4.5



III.1.2. Sludge management situation in Vietnam

- **Wastewater and sludge management**

In Vietnam, a nationwide survey in 54 provinces/cities in 2001 found that 75% of domestic wastewater in large cities and 45% in smaller cities are discharged into sewers (Khai, 2007). In urban areas, the wastewater almost includes wastewater from industries, hospitals and agricultural activities (Phuong et al., 2010a). In recent years, there has been a rapid increase of industrial activities in Vietnam that generate a significant amount of industrial wastewater and industrial sewage sludge (LBCD, 2010). Especially, the big cities such as Hanoi and Ho Chi Minh have experienced a rapid economic growth and urban expansion in the last couple decades. However, appropriate cities planning and wastewater management for both industry and household have not been done. Most of manufactures were established in the 1950s with almost assistance from China. The plants are old and are not equipped with appropriate wastewater treatment facilities (Nguyen et al., 2010). Instead, in many cities, industrial wastewater is sometimes discharged into municipal collection systems when an alternative is not available (De Barreiro & Parkinson, 2005). Industries close to rivers tend to discharge their wastewater directly into these rivers (Scott et al., 2004). These activities caused various environmental problems and required the emergency development of wastewater treatment technologies.

Sewage sludge is mainly treated by burial in landfill areas (Hung et al., 2015). The industrial sludge collected represent about 65% of the total generated sludge mass (Dung et al., 2015). These data only relate to factories registered at the Vietnamese environmental protection agency. The fate of industrial sludge that is not collected by authorities is unknown and it is most likely not treated properly. According to (Thai, 2009), hazardous and toxic wastes from industrial activities are dumped on land, leading to negative impacts on the environmental quality in Vietnam. Thus, in Vietnam, not only wastewater but also sewage sludge necessitates implementation of techniques for treatment and environmental protection.

- **Fertilization in agricultural activities**

Like some Asian countries, Vietnam has a long tradition in the use of wastewater in agriculture (Fuhrmann et al., 2016; Qadir et al., 2010). Reuse of wastewater as nutrient sources has become common practice in Vietnam, especially in peri – urban areas. Wastewater was used for agriculture or aquaculture in 93% of Vietnamese cities with total area estimated 9000 ha (Scott et al., 2004).

In addition, besides the use of chemical fertilizers, farmyard manure (FYM) which is produced from pigs, livestock and poultry are also used in agricultural land (NISF, 2003). Traditionally, Vietnamese households have used solid animal manure to fertilize crops in fields and gardens (Huan et al., 2005). It is estimated that about 75% of the 65 – 70 million tons of FYM produces is used for rice and vegetable, and the rest is applied to other crops such as coffee, tea, peppers, strawberries, etc. For a long time, the biosolid from chicken or pig manures co-composted with rice straw (composted manure) and chicken manure were applied in agricultural at a rate of 20 tones/ha and 14 tones/ ha, respectively (Nguyễn, 2008).



Moreover, in rural area composting buffalo manure (Ngo et al., 2011) or composted human feces (Khoa et al., 2005) are also commonly used as crop fertilizer.

In recent years, the use of sludge from wastewater treatment plant was also noted in some research for supply organic matter and mineral nutrients for crops productions, especially sewage sludge from WWTP, which often contained high amount of C, high ratio of C/N. Sludge from the paper industrial WWTP (Xuan Cu, 2015) in North of Vietnam, and/or sewage sludge from WWTP of industrial parks in South of Vietnam (Dung et al., 2015) are used as fertilizer leading to additional elements in soil such as N, P and organic matter, etc, required for plant growth. However, in most of the studies, the sewage sludge comes directly from the discharged point without any treatment method. Currently, the direct use of municipal for agricultural soil application is not feasible due to its high pollution levels such as high concentration of heavy metals were observed in sludge (Hung et al., 2015). Therefore, municipal sewage sludge in urban areas of Vietnam need to be treated with an appropriate treatment method to reduce pollution level, to produce valuable by- product with low treatment cost.

- **Sludge application on land regulations in Vietnam**

The application of sludge on land for agricultural purposes is strongly regulated at the EU and USA level under the sludge directive, prohibiting the use of untreated sludge on agricultural land, except if they are injected or incorporated into soil (Dung et al., 2015). However, there are no Vietnamese regulations related to this issue due to the lack of sludge treatment stabilization and/or treatment technologies. The Vietnamese legislation (Nation Decree No. 59/2007) and Circular 12/2001/TT-BTNMT (MONRE , 2007, 2011) on solid waste management only specifies that solid waste must be classified, stored, collected, and treated by appropriate treatment methods. These regulations do not provide more details on what is considered as an appropriate treatment method. Current management options for sewage sludge include landfill disposal without treatment; stabilization/solidification prior to landfill disposal, and incineration (MONRE , 2007). At the present, there are no guidelines regarding sludge reuse for agricultural purposes, therefore, no Vietnamese legislation until now for heavy metals limitation on sludge applied on soils. Consequently, no specific guideline exists for water-soluble concentration of heavy metals in waste sludge regulated by the Vietnamese environmental legislation. However, the Ministry of Natural Resources and Environment provides the National Technical Regulation on Underground water quality – NTRUWQ. The concentration of heavy metal can be considered as an indicator for the risk of water contamination caused by on – land application of sludge. Hence, we performed a comparison between the heavy metals concentration in leaching water and the PTEs contents in underground water quality following the National Technical Regulation – NTRUWQ (MONRE, 2008) to evaluate the risk of heavy metals leaching from sludge land spreading.



III.2. Effect of sludge amendment on soil properties and heavy metal bioavailability

Land application of sewage sludge is both environmentally and economically advisable. It provides organic matter (OM) to soil and this addition may represent a good alternative to prevent degradation of soils and to improve many physical properties of agricultural soils such as water holding capacity, aeration, porosity and cation exchange capacity. Moreover, the application of this residue offers the possibility of recycling plant nutrients with the beneficial effects on soil fertility and plant nutrients (Scotti et al., 2015).

As the sewage is treated, it goes through a series of processes that reduce the concentrations of easily decomposable organic materials. The insoluble solid residue remaining after sewage treatment is referred as biosolids, domestic wastewater residuals, or sewage sludge. The safe disposal of the sewage sludge is one of the major environmental concerns throughout the world. Disposal alternatives that have been tried include soil applications of the sewage sludge are suggested to be the most economical sludge disposal methods.

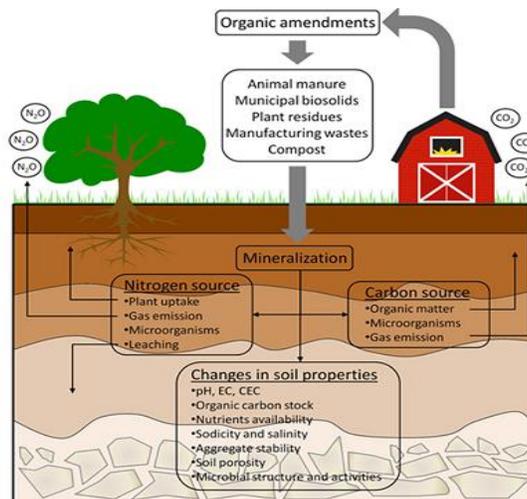


Figure 19: Schematic representation of the effect of organic matter amendments on soils by acting as sources of carbon, nitrogen nutrient and heavy metal leaching (Scotti et al., 2015).

As widely reported in literature, the use of sludge amendments increase soil organic matter (Khaliq and Kaleem Abbasi, 2015; Thangarajan et al., 2013b) consequence soil aggregate stability, water holding capacity and soil porosity, thus improving soil quality (Leroy et al., 2008). As presence in Figure 19, the latter soil properties is strongly correlated to soil organic C, since the addition sludge as one type of organic material amendments normally increase soil organic C and conversely decrease soil bulk density. Sludge can affect directly or indirectly to soil physical properties or heavy metal distribution or mobilization due to sludge stabilization process or sludge characteristics. The interaction of soil and sludge components to heavy metal impacts the heavy metal speciation and leachability.



III.2.1. Effect on nutrient contents in soil (fertilizing value), effect on soil structure (amending value)

Land application of sewage sludge has great advantages - in view of its fertilizer and soil conditioning properties, unless it contains toxic substances. The heterogeneous nature of sewage sludge produced at different treatment plants and variations between seasons necessitates knowledge of the chemical composition of sewage sludge prior to the land application. Characteristics of sewage sludge depend on wastewater treatment processes and sludge treatment. Generally, sewage sludge is composed of organic compounds, macronutrients, and a wide range of micronutrients, non-essential trace metals, organic micro pollutants and microorganisms (Gaber et al., 2011).

Organic matter added to the soil as sewage sludge composts improved the soil properties, such as bulk density, porosity and water holding capacity (Loper et al., 2010). The chemical properties of sludge-soil mixtures not only depend on the properties of the soil and sludge and the application rates of the mixtures, but also on their interaction and soil pH. The effect of sewage sludge application to soil on water retention, hydraulic conductivity and aggregate stability showed that raw, as well as digested sludge increased the total soil water retention capacity with the greatest increase in the raw sludge amended soil (Singh and Agrawal, 2008a). Sludge addition in soil caused a significant increase in soil hydraulic conductivity after incubation. The raw sludge treatment had the highest percentage of stable aggregate of the incubation (Jien and Wang, 2013; Saad, 2016).

III.2.2. Effect on soil pH

The soil physical conditions could be deeply modified by sewage sludge application. An increase in soil pH has been reported in soils applied with municipal sewage sludge (Usman et al., 2012). Lowering of soil pH is also reported (Oleszczuk et al., 2012). The changes in soil pH have been correlated with the calcium carbonate content of sludge and acid production during sludge decomposition. Soil pH consideration is especially important in view of trace metal abundance in sewage sludge (Singh and Agrawal, 2008a).

The changing of pH value when sludge applied on soil also affects the dissolution of metal in soil. Singh and Agrawal, (2008a) assuming that Zn, Cu and Ni behave similarly as pH varies when sludge applied in soils, maintenance of a pH above 6.0 for grassland and 6.5 for arable soil which the sewage sludge is applied were recommended. A study to evaluate the effect of pH on release of Zn, Cu and Ni from the sewage sludge to soil showed that metal concentration released to the supernatant liquid increased as pH decreased below the threshold value, which was 5.8 for Zn, 6.3 for Ni and 4.5 for Cu loaded sludge. The metal content of the supernatant was small and relatively constant above the aforesaid pH values. In speciation experiments, the proportion of soluble Cu present as Cu^{2+} in CuCl_2 was found to be related to pH, whereas the proportion of soluble Zn present as Zn^{2+} was scarcely correlated with pH (Kwon-Rae and Owens, 2009; Pérez-Esteban et al., 2014).



III.2.3. Effect on soil heavy metal content

The metal concentrations in the sewage sludge depend on several factors such as: Sludge origin; Sludge treatment processes, etc. The bioavailability of the sludge borne metals to soil is further influenced by soil properties such as pH, redox potential, and organic matter, as well as sludge application rate (Golui et al., 2014).

Studied on trace element concentrations of sludge evaluates their interactions with soils to determine their effects on plant growth (Bose and Bhattacharyya, 2008; Dharni et al., 2014; Jamali et al., 2009a; Patel et al., 2016). The total concentrations of trace elements in sludge were highly variable depending on the sources, which were related to different industries discharging effluents in the sewage system. Concentrations of Cu, Zn, Ni, Co and Cu were consistently greater in saturation extracts obtained from sludge than those obtained from soils of any sampling sites. The maximum total concentration of Cd, Ni, Cu, Pb, Zn, showed positive correlation with concentrations of these elements found in soil solution (Ashworth and Alloway, 2008). Following the Table 8, the heavy metals concentration in soils following to sludge application often increase comparing with control soils. However, the changing of heavy metal contents depends not only on sludge stabilization process and soil type but also on initial heavy metals contents in sludge or soils (Contin et al., 2015). Besides, in soil, heavy metal elements are distributed in various forms such as solid phases (Silveira et al., 2003b), free ions in soil solution (Singh and Agrawal, 2008a), soluble organic mineral complexes (Scotti et al., 2015), or adsorbed on colloidal particles (M. J. Sánchez-Martín et al., 2007). Sewage sludge addition to soils therefore could affect potential availability of heavy metals. The solubility and consequently the mobility of metals added with sewage sludge are at least in part controlled by organic matter decomposition and the resultant soluble organic matter decomposition is binding or complexing with heavy metal ion and leaching through the soil layers (Egiarte et al., 2006a; Parkpian et al., 2002a; Speir et al., 2003a). Trace metal bioavailability is also dependent on the form of organic matter, i.e., soluble (fulvic acid) or insoluble (humic acid) (Violante et al., 2010). Insoluble organic matter inhibits the uptake of metals, which are tightly bound to organic matter; however, increase the availability by forming soluble metal organic complexes. With stabilization of organic matter decomposition rates, the level of soluble organic matter reduces leading to a reduction in the bioavailability of metals (Quenea et al., 2009; Silveira et al., 2003b).



Table 8: Effect of sludge amended soils on heavy metals (HMs) contents comparing with control soils

Sludge	Soils	Heavy metals (mg kg ⁻¹)								Effects	Refs
		Ni	Cu	Zn	As	Cd	Pb	Cr	Hg		
Composted sewage sludge	Slit loam from farm land area	8.21	24.3	86.8	2.68	0.39	7.21	-	-	Increase	Fang et al., (2016a)
Dehydrated sewage sludge	Loam soil from arable field	17.9	18.7	75.5	-	< 0.2	26.4	14.7	-	Increase	Suhadolc et al., (2010a)
Low metal sewage sludge	Basic loam	31	16	58	-	-	-	-	-	Maintain stable	Toribio and Romanyà, (2006a)
	Acid loam soil	17	13	15	-	-	-	-	-		
	Basic loam soil	6.5	3.2	17	-	-	-	-	-		
Metal-enrich sewage sludge	Basic loam	38	45	123	-	-	-	-	-	Increase	
	Acid loam soil	19	27	61	-	-	-	-	-		
	Basic loam soil	12	24	66	-	-	-	-	-		
Anerobic digested sludge	Suburban soils	0.85	63	89	2.8	0.46	0.047	2.9	0.085	Increase	Qi et al., (2011)
	Industrial soils	11	84	40	0.59	1.5	49	23	0.095		
Composted sewage sludge	Farm land soils	-	846.3	1709.1	-	-	-	-	-	Cu contents does not change and Zn content increase	Gusiatin and Kulikowska, (2015)



III.2.4. Effect of sludge amendment on the fate of trace metal element in soils

III.2.4.1 Mechanisms of heavy metal interactions with soils

To evaluate the potential impact of sludge application to land spreading, it is necessary to understand the mobility and bioavailability of heavy metals in soil. Metal solubility in soils is controlled by adsorption/desorption, precipitation/dissolution and complexation reactions (Figure 20). These interactions influence the partition of metals in the liquid and solid phases, and are responsible for their mobility and bioavailability (Silveira et al., 2003b).

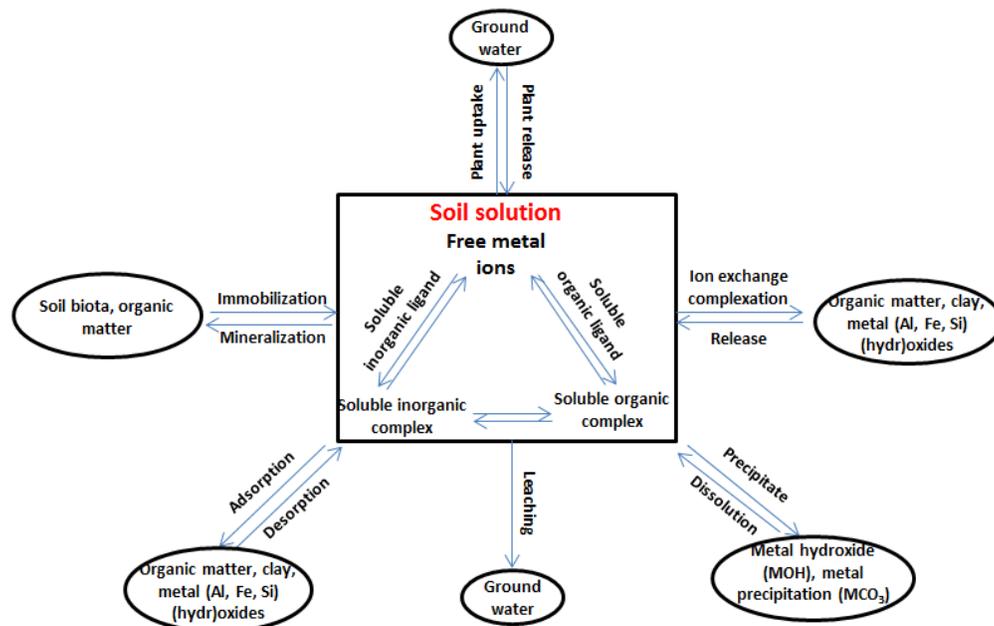


Figure 20: Possible reaction involved in physical, chemical and biological transformation of metal in soils (Seshadri et al., 2015).

Following Figure 20, the general mechanism involved in the transformation of metal ions in the soil led to retention (mediated by sorption, precipitation and complexation reactions) or loss (plant uptake, leaching and valorization) of heavy metals. The lower the metal solution concentration and the more sites available for sorption, the more likely that sorption/desorption processes will determine the soil solution concentration. However, the fate of metal in the soil environment is dependent on both soil properties and environment factors.

III.2.4.2 Experimental methods to study heavy metal speciation on sludge amended soil

Heavy metals in sludge and soils are present in various forms. Therefore, the total concentrations in sludge amended on soils cannot provide a precise index for evaluating their behavior according to soil properties. Many extraction methods were used to study the distribution of heavy metals after sludge amendment. It must be noted that many kinds of trace metals will have different behavior in terms of



distribution which was affected not only by sludge and soil characteristics but also by the extraction method using for heavy metal speciation study (Kunito et al., 2001a)

Over the last decades, scientists have become increasingly aware that determining total concentrations of heavy metals elements in sludge amended on soils provides very limited information about their mobility within the profile, bioavailability and toxic potential to ecological system (Vasile and Tanase, 2010) Nowadays, numerous laboratory studies have reported extraction method for studying the leaching or bio-accessibility of heavy metals either in sludge and soil (separately) or in sludge amended on soil. Single extraction methods are used to provide a rapid snapshot of metal leaching from environmental solids or sludge (Quevauviller and Olazabal, 2003; Sahuquillo et al., 2003). However, sequential extraction or fractionation tests have been the commonly method chosen so as to provide information about regarding detailed insight into different metal – soil association (Bacon and Davidson, 2007a; Filgueiras et al., 2002a; Okoro and Fatoki, 2012; Rao et al., 2008a). The fractionation tests are based on the use of different extractants of increasing elution strength. Hence, the extraction methods allow determining the metal proportions associated to each component of the solid phase and, thus, to predict its solubility and leachability. The sequential chemical extraction procedure has been used especially for determining metal forms added to soil via sewage sludge (Kim and McBride, 2006).

Therefore, fractionation studies are aimed at determining the chemical forms in which metals occur, because metal mobility bio-accessibility and bioavailability strongly depend on their chemical and mineralogical forms (Bacon and Davidson, 2007b; Baeyens et al., 2003a, 2003b; Filgueiras et al., 2002b; Rao et al., 2008b). Sequential extraction protocols individually separate chemical forms of metal species that have different leachability, normally including:

- **The ion-exchangeable fraction**: This fraction reflects the amount of heavy metals that would be released into the environment when conditions become more acidic. Therefore, this fractions will give adverse impact to the environment.
- **Metal bound to Fe-Mn hydroxides**: This fraction represents the concentration of metal bound to iron and manganese oxides that would be released if the substrate was subjected to more reductive condition. Furthermore, a reduction of low pH might have also decrease the re-adsorption of metals from the liquid phase.
- **Organic matter**. This oxidation fraction represents the amount of metal bound to the organic matter and sulfides that will be released into the environment if conditions become oxidative.
- **Residual fraction**: The metals in the residual fraction are strongly bound to the crystalline structures of the minerals. These metals are not easily extracted or removed.

The most widely accepted fractionation tests are the so-called 3-step BCR developed under the auspices of the Community Bureau of Reference (formerly BCR, but currently termed the Standard, Measurement



and Testing (SM&T) Program of the Commission of the European Communities (Rauret et al., 1999; Ure et al., 1993), where bio-accessible metals are associated to the following stages:

- **Stage I:** CH_3COOH extraction – aiming at identification and measurement of assailable and carbonate – bound metals content (F1 or EXCH fraction- exchangeable).
- **Stage II:** $\text{NH}_2\text{OH-HCl}$ extraction – aiming at identification and measurement of the content of metals bounds with amorphous iron oxide and manganese oxide (F2 or INP fraction: reducible or Fe-Mn oxy hydroxides).
- **Stage III:** $\text{H}_2\text{O}_2/\text{CH}_3\text{COONH}_4$ – aiming at identification and measurement of the content of organometallic and sulfide fraction (F3 or OM fraction – oxidizable or organic matter).
- **Stage IV:** mineralization of residual fraction with the mixture of concentrated acid (HCl, HF and HNO_3 – aiming at identification and measurement of the content of metals bound with silicates (F4 or RES fraction – residual).

However, the BCR sequential extraction procedure require an overall operation time of about 48h and it takes around 10h for each extraction steps. This made limitations for BCR extraction method due to time consumption. There has been considerable recent interest in the use of ultrasound to improve the extraction method. A rapid ultrasound accelerated sequential extraction procedure has been reported by Kazi et al, (2006), to develop sequential extraction proposed by BCR protocol. The extraction process by using the compromise of BCR and sonication conditions in ultrasonic bath could be completed in one hour, and the overall metal recoveries were 95-100% compared to the conventional BCR protocol. Recently, the method complexing BCR and ultrasonic was used widely for heavy metal extraction.

In addition, because of the different and complex distribution patterns of metals among various chemical species or solid phase some sequential chemical extraction methods were developed (Jamali et al., 2009b). The two reagents validated by a group of European researchers coordinated by the Measurements and Testing Program of the Commission of the European Community, in single – extraction procedure (Sahuquillo et al., 2003), are EDTA 0.05 M and CH_3COOH 0.43 M. The EDTA, in either the disodium or di-ammonium salt form, has been used extensively as an extractant of available potentially heavy metals, neutral salt extractants are generally weaker than EDTA, and give an indication of the available immediately metals (Beckett, 1989). In some trials, EDTA was found to give a very good indication of the pollution hazard of heavy metals in soils as well as being a reliable test for predicting plant – available metals.

However, the classification of chemical forms of metal species by sequential extractions is usually performed by batch wise methods with tedious and time-consuming unit operations such as filtration, centrifugation and reagent replacement. Those manual operations are likely prone to sample contamination, and they often limited data accuracy.



III.3. Heavy metal distribution in sludge amended soils

III.3.1. Heavy metal distribution according to the chemical speciation in soils

Distribution and leachability of heavy metals is significantly influenced by various natural processes in the sludge - soil-water-plant system, such as sludge application rate, microbial activities, weathering, drainage; etc. All these processes are interactive with the potential to change properties of soil, thereby influencing the distribution and leachability of heavy metals. When sludge is applied to soil, it can increase the content of soil organic carbon and microbial activities for example (Aranda et al., 2015). The enhanced microbial activities resulting from sludge land application are factors influencing leaching of heavy metals.

To date, a variety of studies have focused on the effect of sludge land spreading on the leaching ability and speciation of heavy metals in the amended soil. Doelsch et al., (2010) determined the organic matter mineralization of the soil amended by pig slurry sludge and composted sludge during 28 days and assessed its impact on the leachability of heavy metals. Their study highlighted the Cu binding ability of organic matter from composted sludge amended soil. Part of the exchangeable Cu fraction was quickly complexed by the organic material and the exchangeable heavy metal fractions in both sludge amendments were less than 10%.

Rajaie et al., (2006) explored the change of Cd speciation in the municipal composted sludge amended soil during 16 weeks. Their results showed that, about 82-88% of Cd content in treated soils was converted to exchangeable, carbonate-bound and organically bound fractions. These fractions presented high Cd bioavailability in soil sludge amendments.

Pardo et al., (2011a) investigated how the speciation of heavy metals changed when composted sludge were applied to soil after 56 days. Following this study, while the addition of composted sludge led to an increase in NaOH-extractable Cu concentration (associated with organic matter Cu fraction), the soluble and exchangeable Zn and Pb fractions decreased. They found that, the increase in soil pH by the application of composted sludge was the main responsible for the changes in metals fractionation in the soil. Also, the CO₂ produced during mineralization of the organic composted sludge amendments could have promoted the formation of ZnCO₃ and PbCO₃ in the carbonate extractable fraction.

Jalali and Arfania (2011) study the distribution and fractionation of Cd, Cu, Pb, Ni and Zn in municipal sludge amended calcareous sandy soil. They indicated that when sludge was added to the soil, the speciation of all heavy metals changed due to sludge characteristics.



Table 9: Effect of sludge amendment on soil heavy metal distribution

Sludge amended on soil	Heavy metal	Control soils	Land spreading of sludge on soils	Refs
Municipal sewage sludge	Cd Pb Ni Zn Cu	RES>OM>INP>EXCH: for all metals elements	Pb: OM>EXCH>RES>INP Ni: OM>EXCH>RES>INP Zn: RES>INP>OM>EXCH Cd: OM>EXCH>INP>RES Cu: RES>OM>INP> EXCH	Jalali and Arfania, (2011)
Dried sewages sludge	Cd Cu Pb	Hapludoll soil: Cu: RES>OM>INP>EXCH Pb: RES>INP>OM;EXCH Cd: Not detectable Natraquoll soil: Cu: INP>RES>OM; EXCH Pb: INP>RES>OM; EXCH Cd: Not detectable Arguidoll soil Cu: OM>INP>RES>EXCH Pb: INP>RES>OM; EXCH Cd: Not detectable	3 soils presented similar trend for 3 elements: Cd: RES- (EXCH, OM, INOR: Not detectable) Cu: RES>OM>INOR>EXCH Pb: RES>INOR>OM- (EXCH-no detectable)	Torri and Lavado, (2008)
Dried and composted sewage sludge	Cu Zn	Cu: RES>OM>INP>EXCH Pb: RES>INP>EXCH>OM	Cu: OM>RES>INP>EXCH Pb: RES>INP>EXCH>OM	Kunito et al., (2001a)

(RES: residual fraction; OM: organic fraction; INP: inorganic precipitates or Fe-Mn oxy-hydroxide; EXCH: exchange fraction)

Kunito et al., (2001b) found that the sludge application increased the percentage of Cu in the NaOH fraction. The sum of the three bioavailable fractions $\text{KNO}_3 + \text{H}_2\text{O}$, NaOH, and EDTA-Cu, exceeded 50% of the total Cu content in all the soil samples. In case of Zn, the sludge application increased the percentage of Zn in EDTA fraction. The percentage of Zn in the $\text{KNO}_3 + \text{H}_2\text{O}$ fraction was also higher in the control soils. These results indicated that:

- The ratio of bioavailable fraction to the total amount was large for Cu,
- The ratios of the most labile fraction, the exchange fraction, to the total amount of Zn were extended when sludge was amended on soils.



In addition, distribution of metal in different fractions appeared to be different when anaerobic and aerobic digested sludge was amended on soils (M. J. Sánchez-Martín et al., 2007). Most of the metal was concentrated in the residual fraction with percentages ranging from 60 – 80% for Cu, Pb and Zn and 80% for Cd, Cr and Ni. High percentages of heavy metal in residual fraction concerned immobilization of metal elements in soil after amendment. In general, the most important changes in distribution fractions were observed by changing the linking between the heavy metal and soil components which was impacted significantly by type and rate of sludge amendment (M. J. Sánchez-Martín et al., 2007).

III.3.2. Heavy metal distribution according to the soil depths

The enrichment or distribution of regulated metals in near-surface soil following sludge application, in relation with soils depths, has been reported in various types of soil.

Pardo et al., (2014) showed a decreasing heavy metal gradient from near surface soil (0-30 cm) to deeper soils depths (30 -70 cm, 70 – 100 cm) when mine soil was amended with composted sludge. Soil with high clay content could preferentially retain regulated metal, which may explain the accumulation of regulated metal in surface soils. In mine soils, several anions such as chloride and sulfite, which can complex or associate with metals ions, reduced the metal solubility and mobility. Metals that could forms complexes or precipitates with anions were more resistant to leaching and thus were retained in the near surface layer after entering into the soils (T. Pardo et al., 2014a). It shows that sludge application may have more impact on the accumulation of metals with lower mobility in soils than of metals with higher mobility. It is particularly noticeable in areas like those assayed where erosion rates are low.

Similar, Jalali and Arfania (2011) study the distribution of Cu, Cd, Pb and Ni in a calcareous sand soil receiving municipal sludge waste. The heavy metal distributions at each soil layer were analyzed by diethylene-tri-amine-penta-acetic acid (DTPA) extractable method (0.005 mol of DTPA, pH 7.2, 1:10 w/v ratio). This extraction method showed that a significantly greater concentration of DTPA extractable Cd, Cu, Pb, Ni and Zn was found in soil receiving sewage sludge. Most of the heavy metals were distributed in the top 5 cm soil layer and the heavy metal concentration decreased with soil layers. Cd mostly accumulated in the 0 – 2 cm soil layer, and there was a difference among treatments in Cd concentration in the 0 – 10 cm depth. Zn followed a similar pattern of high accumulation as those of Cd in the 0 – 1 cm soil layer and low in the next layer. Pb mostly accumulated in the 0 – 5 cm soil layer and there was a difference among sludge amended rate and Pb concentrations decreased dramatically in the 5 – 20 cm layer. The Ni concentration in all layers in both treatments was different in the 0 – 1 cm soil layers and similar in the rest of the soil layers and more than that of the control soil. In the 0 to 5 cm soil layers, the proportions of the metals in the exchange fraction followed the order Pb> Cd > Ni> Cu> Zn (Jalali and Arfania, 2011).



III.4. Heavy metal leachability in sludge amended soils

In the previous part, some studies done on field sludge application sites have concluded that metals are strongly bound in the topsoil, since little or no metals accumulate in the soil below the depth of incorporation (Egiarte et al., 2006a).

Hence, adding sludge on land may mobilize or immobilize heavy metals leachability. The heavy metal bioavailability depends on not only sludge characteristic, rate of application and heavy metal pieces but also soil properties. As mentioned above, heavy metals can bind to soil through the processes of ion exchange and chemisorption. Oxides of Fe, Al and Mn can provide chemisorption sites for metals.

III.4.1. Method to study heavy metal leaching behavior on sludge amended soil

Many researchers have been developed for the evaluation of leaching from waste sludge amended soil that provides specific leaching test methods and the approach to testing and evaluation (Kosson et al., 2002). Almost these tests take under consideration the utilization scenario, and consist of either batch equilibrium or column tests.

III.4.1.1 Batch tests

The most of the bioleaching studies for heavy metal bioavailability on sludge amended soil have been reported using laboratory batch test scale which are easy to operate and are conventionally used to generate data required for development of the process before larger – scale application.

Normally, the bioleaching process was also found to be efficient at higher sludge solids content or low L/S ratio. This clearly suggests that at higher sludge or soil solid concentration more solid phase can be treated at a given volume of batch and time. However, a decrease L/S ratio of sludge soil solid can lead to a decrease in metal solubilization. A high solid contents leads to high buffering capacity and increase the organic content of in the solid phase (Liu et al., 2007). Further, the low L/S ratio affects the oxidation – reduction potential (ORP) values of leaching solution. The lower ORP is achieved at low L/S ratio resulting in solubilization of metals (Ryu et al., 2003).

In the literature, heavy metal leaching in sludge was assessed under different L/S ratio from 100 to 5 L/kg Kim et al (2005), and from 0.1; 0.2; 0.5; 1, 2, 5, to 10 (L/kg) (Naka et al., 2016a).

In some studies, authors observed a real threshold in leaching as a function of L/S ratios. (Naka et al., 2016b) measured clear difference between Cr, Pb, As, Se and Cu concentration in leachates at an equilibrium time at L/S ratio lower than 1 L/kg. They also found that the electrical conductivity and concentrations of Cr, Pb, As, Se and Cu in the leaching solutions appear to have the same pattern at L/S ratios higher than 1 L/kg.

In other study, the leaching was not dependent. In the study of Alabed et al., (2008), static batch tests using deionized water was performed on sludge at L/S ratios of 5, 10, 20, 50 (L/kg). At the end of



experiment, Cu and Zn solubility were independent of the L/S ratio. Cu released increased as the L/S ratio increased from 5 to 10 (L/Kg). At L/S ratio of 10 (L/kg) Cu and Zn leached to maximum concentration in leachates. A moderate increase in As and Se release was observed with increasing L/S ratio, with the leached amounts less than 10% of the total metal concentration.

Hence, the increase of L/S ratio, the high amount of the liquid phase results in some dilution of the metal concentration. This increase in extract concentration with increasing L/S ratio suggested dissolution mechanism (Kosson et al., 2002; Sanchez et al., 2000; Tiruta-Barna et al., 2004). This is mainly due to the agitation conditions and dilution effects in both testing regimes, since the concentration of the constituents in the material solution are not only dependent on the total amount, but also on the available amount and the release kinetics.

Batch tests are usually used to determine the soluble particulate phase partition K_d . Distribution coefficient K_d indicated the heavy metal distribution and bioavailability in batch test experiments. The K_d is a useful parameter for comparing the sorption capacities of different soils or materials for any particular ion, when measured under the same experimental conditions. The distribution coefficients (K_d) are calculated as the equilibrium adsorbed element concentration normalized to the equilibrium element concentration in solution (Shaheen et al., 2013), where the equilibrium adsorbed element concentration is given per unit weight of soil and the equilibrium element concentration in solution is given per unit volume of liquid.

The distribution of heavy metals between the solid phase and solution phase is an index of a metal's potential mobility and retention of these elements in soils or sludge amendment (Rennert and Rinklebe, 2010; Rinklebe and Du Laing, 2011; Zhong et al., 2011). The K_d represents the net result of various processes by which element ions can be transferred between the solid phase and solution phase and are satisfactory for comparing the behavior of different soils with respect to a given cation under given condition. It is especially useful when the irregularity of empirical sorption and/or desorption isotherms hampers or prevents the fitting of simple empirical curves or theoretical models such as Freundlich and Langmuir isotherms, as is often the case when the presence of more than one element results in competition for sorption sites. The higher the K_d of a metal the stronger its sorption and retention onto the soil solid phases by various chemical reactions, and the lower its solubility. In contrast, a low K_d value indicates that a high amount of the element remains in the solution (Anderson and Christensen, 1988; Covelo et al., 2004; Shaheen, 2009). Also, the sorption selectivity sequence of an element by soils could be established at selected values of K_d to obtain one comparable value for each element and each soil.

III.4.1.2 Column test

This method can be used for assessing the impact of contaminated materials on the soil – groundwater pathway, since the percolation-based data they provide is the result of a flow-through similar pattern to that found in field condition. Column tests can account for contaminant wash out at lower L/S ratios and the changes in solubility controlling phases that occur as a result (Dijkstra et al., 2006). The correlation between batch and column tests has been studied, usually finding a good correlation between both types of testing.



There are many column works which investigated the accumulation of trace HMs in soil caused by sludge amendment, focused on the metals retention and mobility related to the different techniques methods (column materials, design; solution irrigation and flow rate of solution, etc). The column experiments were established based on the study purposes and laboratory conditions and the HMs selected were chosen either due to their high leaching potential, their intensive application or due to their (eco) toxicological potency. In order to have generalized data and repeatable experiments, the research is conducted on an artificial model soil according to internationally accepted guidelines had to be performed. The column experiments were prepared following to organization for Economic Cooperation and Development (Chemicals, 2005), which normally is considered to improve the understanding of the distribution, fate and impact of HMs in the environment (Pontoni et al., 2016b). Moreover, in column experiments, test conditions can be controlled to closely simulate flow characteristics in the field. The test consists of percolating the heavy metals transfer through the column filled with soil and/or sludge amended soils. Concentration heavy metals variation and physico-chemical parameters in leachates are typically monitored at the different levels along the column height and manuscript of the experiments for the study the fate of heavy metals leaching through soil column. Samples of solutions from effluents are collected and subjected to chemical analyses to determine heavy metal concentration changes and to obtain physico-chemical impacting parameters such as the pH, redox potential, end electric conductivity, dissolved organic matter contents, etc., in leaching solutions.

pH: could affect the capacity of soils in maintaining and releasing the heavy metals, since it play an important role in adsorption/desorption, and ion-exchange processes (Begum et al., 2013; Cao et al., 2001; Polettini et al., 2007; Zou et al., 2009). The pH values of leachates reflexed the interaction between heavy metals and proton exchange during leaching experiment. The responses of pH leachates were different for sludge application, soil characteristics, leaching experiments and heavy metal study, etc.

Redox- potential (ORP): Redox potentials measured in the leaching solution with the electrode vary from negative to positive values (Kumar et al., 2013). Oxidization process in soil column was normally presented by ORP values. Other processes in soil column such as: biological degradation of organic matter, a variety of biological and abiotic redox processes of dissolution/precipitation of minerals, complex formation, ion exchange and sorption also affect to the ORP values in leachates and reflex the heavy metal bioavailability (Bjerg, 2014). Generally, when ORP values decrease, the heavy metal leaching ability also decrease (Cappuyens and Swennen, 2008).

Electrical conductivity (EC): The EC indicates the content of salts in solution and is often used as a measure of ions changes in soil column (García-Figueruelo et al., 2009; Huang et al., 2006; Zhou et al., 2005). Salinity changes related to ion exchange can be estimated by the measurement of EC in the leachate during simulated rainfall for column experiment. Normally, high EC value of leachates indicated high heavy metal content leaching ability (Kaschl et al., 2002).



Dissolved organic carbon (DOC): The leaching of DOC depended on the organic matter (OM) content of soil sample that were packed in columns. i.e. higher OM yielded higher DOC in the column and leaching solutions (Kumar et al., 2013). However, under the columns experiment, the DOC contents in collected leachates can increase or decrease according to the leaching experiment conditions. The DOC can complex with heavy metal and leaching together through soil column. It is important parameter indicating the fate of heavy metal leaching ability on soil columns

III.4.2. Effect of soil characteristics on heavy metal distribution and leachability

III.4.2.1 Soils mineral and organic matter composition

Research on the transfer of heavy metals from soils to water or plants following sewage sludge amendment has been focused on evaluating the ability of surrogate methods (mainly chemical extractants as mentioned above) to assess heavy metal bioavailability in soils (Soriano-Disla et al., 2010), under different sewage sludge rates and availability of heavy metals (Antoniadis et al., 2010). However, a particular metal can behave entirely differently in different soils (McBride, 2003). According to the Table 10, soil properties played an important role in the toxicity and bioavailability of heavy metals. (Cesar et al., 2012a) found higher levels of toxicity were detected for the ferralsol. The abundance of 2:1 clay minerals, high fertility and more basic values of pH seem to be very important in the reduction of toxicity levels for microorganisms in the soils. In some cases, higher contents of nutrients in chernosols may have influenced such heavy metals bioavailability processes (Cesar et al., 2012a).

In addition, it is now widely recognized that the toxicity and mobility of these pollutants strongly depend on their specific chemical forms and on their binding state (precipitated with primary or secondary minerals, complexed by organic ligands, and so on). Soil physical-chemical properties, especially pH, CEC, organic matter and clay content are likely to assume great importance in determining heavy metal elements behavior (Torri and Lavado, 2008).

Heavy metals are often highly persistent in soil, with residence times as long as thousands of years (Alloway and Jackson, 1991). Metals applied with sewage sludge may be retained in the soil as a result of their adsorption on hydrous oxides, clays, and organic matter, the formation of insoluble salts; or the presence of residual sewage sludge particles.



Table 10: Effect of soil characteristic on heavy metal distribution, speciation and leachability when sludge amendment

Sludge amendment	Soils	Impact of heavy metal distribution and speciation	Refs
Dehydrated sewage sludge	Alluvial agricultural soil	Heavy metal distribution did not significantly change after sludge amendment due to higher organic matter content of soils	Suhadolc et al., (2010a)
Drinking wastewater treatment residual	Calcareous and Sandy soils	Sandy soil has higher Cu-residual fraction than calcareous in sludge amended soils due to higher soil carbonates in calcareous soils increasing leachability of heavy metals	Elkhatib and Moharem, (2015)
Centri-dried sewage sludge	Hapludoll, Natraquoll and Argiudol (3 types of Mollisols)	Cu-organic matter fractions in the amended soils followed the sequence Hapludoll>Argiudoll>Natraquoll due to the highest organic components on Hapludoll soils	Torri and Lavado, (2008)
Centrifuged sewage sludge	3 types of soils (loam; clay and acid loam soils)	Acid loam showed the highest metal concentrations in leachates and the basic clay showed the lowest; Low clay content of the basic loam soil may result in increases of the leaching of both Cu and Zn as compared to the basic clay soil due to lower organic matter, clay, and CaCO ₃ content of this soil.	Toribio and Romanyà, (2006b)
Sewage sludge	Ferralsols and Chenosols	Ferralsols are more acidic than chenosaurs leading higher heavy metal leachability when sludge amendment	Cesar et al., (2012a)

Soil pH: Moreover, soil CaCO₃ has often been found to increase soil metal retention, thus, in basic soils metal persistency is expected to be generally very high. A number of studies on acid soils have examined the leaching of heavy metals in sewage sludge amended soil columns. While some studies have shown that the migration of heavy metals is negligible (Parat et al., 2007; Song et al., 2014), other studies have demonstrated that significant amounts of Zn, Cr, Cu and Cd (among others) were readily leached (Suhadolc et al., 2010a; Toribio and Romanyà, 2006b). It therefore concluded that soil properties such as organic matter and soil pH may have a major effect on metal mobility. The addition of sludge may also affect metal mobility, for instance, by increasing soluble organic matter and heavy metal mobility may be enhanced, especially in sandy soil of high pH (McBride, 2003).



However, the evaluation of the transfer of several heavy metals occurring together (e.g. in sewage sludge) to water, to plants, and animal or human, from various soils amended with sewage sludge has received, comparably, little attention. These studies have commonly involved a set of sludge soils, under a range of conditions often restricted to sludge effects in the heavy metals distributions (Basta et al., 2005), the study of the soil characteristics (mineral component, geochemistry, soil texture and colloids, etc) that are primarily responsible for limiting metal uptake at soil or sludge amended soil still needs further research.

Soil organic matter: also plays a key role in complexing and retaining metals. Both solute and in-solute of organic matter form complexes with metals by exchange and chemisorption reaction and hold the metal very strongly (Jalali and Arfania, 2011). Cu and Pb in soil form stronger complexes with surfaces and soluble organics than do Cd and Zn. This tends to make them less mobile in soils, but also provides a greater opportunity for facilitated transport by soluble organics (Jalali and Arfania, 2011).

Dissolved organic matter (DOM) has a significant role in the sludge – amended soil for heavy metal leachability. For example, sludge amended soil increase DOM leaching; this DOM had a significant role on the mobility of Ni in the sludge with 7.7% total Ni was mobile through the soil column (Ashworth and Alloway, 2004). This value was clearly much greater than for Zn and Cu. Thus, of the three metals, Ni was the least well retained by the sludge and is therefore likely to be the most significant environmentally. Its apparent lacks of adsorption to soil solids (once released from the sludge), and its mobility in association with DOM, further suggest the potential for environmental impact (Ashworth and Alloway, 2004).

III.4.3. Effect of sludge origin on heavy metal leachability

Leaching of heavy metals in soil can be explained by various mechanisms such as complexation, precipitation, bioaccumulation, etc. These last mechanisms are also depending on sludge origin and stabilization processes.

Most of the time, the fate of HM in soil is investigated with a specific doping of the soil solution rather than a real sludge land spreading. Then the fate of HM is explained through interactions with organic or mineral matter of the soil solution only. In real conditions, the change in sludge composition and amended soil condition over time influence the chemical forms of the heavy metals and exert a significant control on the release of metal (Sastre et al., 2001). It is important to know whether sludge-borne metals become increasingly available (as organic binding sites are lost through microbial degradation), or do they form more biologically inert species, which are predominantly inorganic in character. Regarding this aspect there is an important lack of information (M.J. Sánchez-Martín et al., 2007).

The total heavy metal amount in sludge amended soils is distributed over some fractions which can be determined as described in paragraph (Figure 20: Possible reaction involved in physical, chemical and biological transformation of metal in soils (Seshadri et al., 2015). The soluble exchange fractions are the most important associated to groundwater pollution and to plant nutrition. The extractability of metals can be reduced many years after sludge application, and this behavior may result from the increase in soil



organic matter content. Therefore, the distribution, mobility and bioavailability of metals are related to their solubility, of their geochemical form and especially depend on sludge application (Silveira et al., 2003a).

For example, the chemical flocculation treatment of sewage sludge with FeCl_3 reduces heavy metals availability in neutral soils amended with anaerobically digested sludge, but markedly increases their solubility and bio-availability in acid soils (Contin et al., 2015).

Cu, Zn and Ni derived from the sewage sludge were found to leach readily from the soils when sludge amended. This fact may be related to the effects of the newly added organic matter and/or sludge fine mineral particles. Theodoratos et al., (2000) found that after the addition of municipal sewage sludge to a soil polluted with Pb, Zn, and Cd, both the leachability and toxicity of these metals was reduced. Similarly, Kiikkilä et al., (2002), after mulching a Cu polluted organic boreal forest soil with an array of organic materials (sewage sludge, compost and garden soil) in a microcosm study, found that Cu mobility was reduced by the newly added soluble organic matter, although the microbial activity of the soil did not change.

Egiarte et al., (2005) studied the leaching of heavy metal derived-enrich anaerobic municipal sludge amended soil. They found that the sludge amendment caused a decrease in leached concentration of Cd. Besides, the municipal sludge used in this study contained 208 mg/kg of Ni, and produced an increase in Ni leachability throughout the whole soil column. This sludge also contained 456 mg/kg of Cu and caused a decrease in Cu concentration when sludge was amended on soil columns. The decrease in concentration of these heavy metals below original levels in the upper 4 cm of the soil column indicates that native heavy metal was mobilized. This phenomenon was probably due to binding reactions with organic and inorganic ligands which were added by the sludge. In deeper horizons, it precipitates forming complexes with organic matter, of a low carbon/metal ratio. For the same reasons, sludge amended soil caused a decrease of Pb concentration while less than 1% of Pb concentration was collected in the leachates. However, sludge amendment did not cause any increase in Cr concentration, only 1% of the Cr added was collected in the leachates.

In other study of Egiarte et al., (2006a), soils were also amended with anaerobically stabilized sewage sludge, under highly acidic condition and very high dose of sludge applied to the soil column: trace metal element like Zn, Cu, Ni, Cu and Pb leached at low percentages and prevented toxic effects.

Moreover, Zn compounds are relatively highly leached from limed sludge amended soil although this metal precipitation may occur at high Zn concentration and at high pH values. This result can be mainly understood by mechanisms of retention in soils through exchange reactions. One possible explanation for this phenomenon is the inability of Zn to compete for exchange sites in the presence of large amounts of Ca added in the lime sludge.

Composting and anaerobic digestion can be considered as stabilization processes having a low short term risk of metal mobilization when applied to soil (Alvarenga et al., 2015).



III.4.4. Effect of sludge application rate on heavy metal leachability

Adsorption, accumulation and distribution of heavy metals may vary between different crops and at different levels of sewage sludge amendments (Singh and Agrawal, 2008b). Several leaching ability of heavy metals have been reported at different rates of sludge amendments in soils (Carbonell et al., 2009; Guo et al., 2012). Singh and Agrawal, (2010) reported that sewage sludge amendment from 0, 30, 45, 60, 90, 120 ton DS/ha modified the physico-chemical properties of paddy land soil, thus increasing the availability of heavy metal in soil with consequently higher accumulation in plant parts. At the rates above 45 ton DS/ha, heavy metal leachability significantly increased. In their study, soil pH decreased, EC increased according to application rates. The decrease of soil pH with higher organic matter and moisture may have increased the heavy metals availability and further uptake by plants during their study.

Higher heavy metal availability in soil due to an increase of sewage sludge application rate is of great concern due an enhancement of leaching leading to nearby water contamination.

In addition, Carbonell et al., (2009) applied stabilized anaerobic sewage sludge on un-treated grass land soil at three application rates: 30, 60 and 120 ton DS/ha for assessment the fate of heavy metals during 21 days experiment. The leaching ability of heavy metal was assessed by their concentration analysis in leaching solution. According to their study, the leachability of heavy metal presented different phenomenon from beginning to the end of experiment at different sludge application rates.

At the beginning of the experiment the highest concentration of Zn was observed in the leachates at the highest application rate. Ni, Pb and Zn concentrations did not show differences between control or sewage sludge amendment soils at every rate. The concentration of Cr showed a significant decrease in leachates with respect to the control values at the lowest and at the highest application rate. On the contrary, Cd and Cu concentration showed a significant increase at the highest application rates. Hg concentrations in sewage amended soil were dose related at any application rate.

At the end of their experimental period, a significant increase of Cd concentration was observed at the lowest and the highest application rates. Cr, Ni and Pb presented similar concentrations for control or sewage amended soils. The largest concentrations were observed for Cu and Zn showing significant differences at the highest and at medium application rates for Cu and at the highest application rate for Zn.

In other hand, moderate dose of sewage sludge (30-60 tons fresh sludge weight per ha) applied to agricultural soil did not produce an increase in heavy metal content, but high does (120 tons fresh sludge weight per ha) caused a significant increase in Cd, Cu, Zn and Hg (Li et al., 2007). Therefore, the metal concentrations of soil solution normally reach to high contents as sludge mass loading increases.



By the way, the application rate of anaerobic digested sludge from 50 t DS/ha up to 100 t DS/ha in the soil caused an increase of Zn in the leachate and in the shoots of Chinese cabbage (Hseu, 2006). The increase of heavy metal leachability may due to the application of in organic matter from sludge amendment.

Tripathi and Tripathi, (2011) showed the soluble heavy metals contaminants up to toxic levels at high sludge application rate of 150 t DS/ha due to the increase the solubility of organic matter mineralization during microorganisms' activities.

However, Somasundaram et al., (2012) reported that at application rates up to 476 tons DS/ha, heavy metal remained mainly in the zone of incorporation, even during long term application. In addition, at application rates less than 100 tons DS/ha, essentially no movement of heavy metals was observed. Gusiatin and Kulikowska, (2015) had the similar assessment when they amended composted sludge on soil at the application rate of 600 tons DS/ha. Their results indicated that the metal transformations in the amended soil proceeding longer than in the non-amended soil (Rigby et al., 2016). Method to study heavy metal leaching behavior on sludge amended soil.



III.5. Conclusions

This bibliographic synthesis thus made it possible to make an inventory of the current knowledge on the evolution of the concentrations and the fate of the heavy metals during the wastewater treatment by conventional or granular sludge and following the sludge stabilization process. We found the great number of studies which focus on HMs adsorption, distribution on individual conventional/granular sludge, while the evaluations of HMs adsorption ability on both of conventional and granular sludge are not regulated and considered.

The literature analysis has led to the conclusion of lack knowledge about the fate of the majority of HMs during different sludge treatment stages and also during spreading, especially in case of granular sludge. Although the effects of sludge land spreading on heavy metal distribution in soils have been widely studied, the influence of sludge characteristics has been rarely investigated. Sludge characteristics can be impacted by sludge stabilization processes and nature of wastewater treatment (i.e conventional activated sludge systems/granular sludge systems). Almost researches focused on conventional activated sludge from wastewater treatment plant. In the range of our study, there is no research regarding after granular sludge land spreading. Therefore, the impact of granular sludge amended soil on heavy metal distribution and leachability was limited. In addition, literatures indicated that the determination of heavy metal leaching behavior and speciation of sludge amended on soil was affected by various parameters which was controlled by not only sludge characteristics but also references of soils and was analysis by different extraction methods. Conventionally, these extraction methods were chosen base on the objectives following the study purposes.

Furthermore, literature synthesis brings a lot of useful information and a better understanding of the mechanisms involved in studying the behavior and distribution of HMs on stabilization of conventional/granular sludge (thickening, liming and anaerobic digestion, etc.) before and after spreading on soil. It is part of this thesis works.

This work focuses on understanding the mechanisms governing the fate of HMs pollutants in the conventional/granular sludge and sludge amended on soils. The aims are to evaluate the different sludge treatment process in terms of either their potential to eliminate HMs or bioleachability in soil during sludge amendment. The understanding of the heavy metal bioavailability on sludge and sludge amended soils mechanisms will couple with the monitoring and analyzing the HMs mobility and impacting factors in leaching solutions. Moreover, the determination of HMs distributions on different soil after and before sludge amendment also indicated the HMs transportation/behavior of HMs on soil.



Part 2: MATERIALS & METHODS

Part 2: MATERIAL & METHODS

I. Description and origin of sludge

I.1. Conventional activated sludge

The conventional activated sludge processes used in this study were taken at three wastewater treatment plants in France (Figure 21).



Figure 21: Location of three WWTPs in France

I.1.1. Sludge of the Limoges wastewater treatment plant 1 (WWTP1)

The different types of sludge used in this thesis: digested, centri-dried, filter-pressed, limed and thickened sludges were carried out from the municipal wastewater treatment plant 1 (WWTP1) in the city of Limoges. This WWTP is an activated sludge plant with a capacity of 285,000 inhabitant equivalents, mainly treating urban wastewater and receiving discontinuously effluent from the nearby municipal abattoir wastewater treatment location (less than 10% of the total incoming volume). The sludge used during the thesis was taken at different stages of sludge treatments (Figure 22).



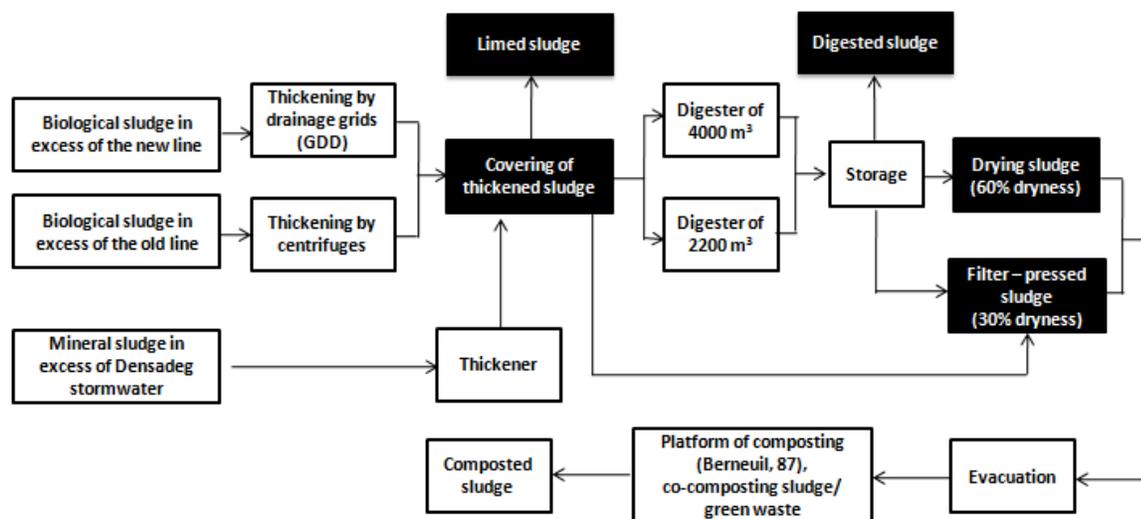


Figure 22: Summary of sludge treatment processes in WWTP of Limoges. In black boxes: sludge sampling.

The sludge treatment process consists on different stages of from activated sludge which was taken from the aeration basin (Figure 22) to stabilized sludge: sludge was thickened by drainage grids (GDD) and centrifugation, then the thickened sludge was stabilized chemically by liming and biologically by anaerobic digestion.

A portion of the thickened sludge is used to perform a chemical stabilization treatment liming. This step consists in adding a quantity of lime (powder) corresponding to 30% of the quantity of dry matter of the thickened sludge, with slow stirring to homogenize the mixture.

Digested sludge was continuously stabilized by physical methods. On one hand, 60% dryness of digested sludge was dried and concentrated by a centri-dry process. On other hand, digested sludge combined with thickened sludge was stabilized by filter-press to ensure 30% of dryness.

More details of sludge stabilization methods were described in (Lachassagne, 2014).

Each sludge sample was taken less than 24 hours before the start of the analysis and the sludge samples were stored at 4°C before manipulation in order to limit the evolutions of the sludge characteristics due to prolonged storage.

Finally, the different stabilized sludge is spread on laboratory scale in soil column tests to assess the leaching potential of copper in soils.

1.1.2. Sludge from WWTP2

Centrifuged and composted sludge was taken at the WWTP2 which is located in urban area. This WWTP handles 49,000 population equivalents and operates in two basic stages of primary and secondary treatment processes. Primary treatment consists of four lines of a screen, flocculation, disordering and



degreasing. In the primary stage, the physical gravity decantation was applied in dry weather and flocculants were added in rainy weather as physico-chemical treatment method. Then, the wastewater was transported to four sequential batch reactors (SBR). This biological treatment was operated with hydraulic and organic loading rate of 15600 m³/day and 2940 kg BOD₅/day, respectively. At the end of processing, the secondary and primary sludge were mixed and dewatered by double centrifugation to constitute centrifuged sludge. Sewage sludge was stored and evacuated two times per week to the composting center. The characteristics of the composting platform were shown in Figure 23.

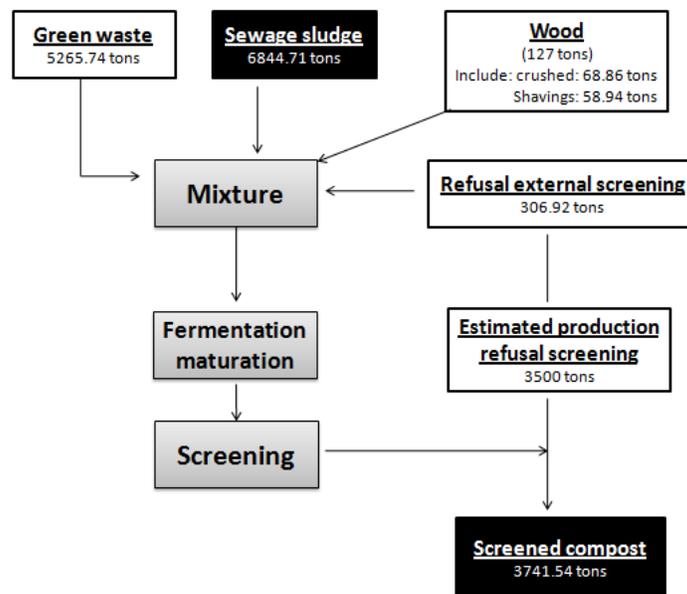


Figure 23: Diagram of composting stabilization process at composting platform of Cahors

I.1.3. Sludge from WWTP3

The wastewater treatment plant-WWTP3 was run with activated sludge in prolonged aeration. The hydraulic and organic loading rates are 225m³/day and 90 kg BOD₅/day, respectively. The treatment comprises 4 sludge drying reed beds (SDRBs) for a population of 1500 inhabitants. Sewage sludge from WWTP3 was stored and hydrated on four SDRBs with total area of 300 m² (75m² for each SDRB). The depth of the sludge layer was about 1.83 m. Then, sludge was sampled according to the quartile method in the reed bed of fours SDRB systems.

I.2. Granular sludge

I.2.1. Anaerobic granular sludge

Anaerobic granular sludge was collected from a full scale UASB reactor treating paper mill wastewater (paper company, France).



1.2.2. Aerobic granular sludge

Aerobic granular sludge created on lab-scale sequencing batch reactor (SBR).

- **Description of experiment setup**

The experiment device consists of identical sequential batch reactor in cylindrical glass column as Figure 24. The working volume of reactors is 1L. The inside diameter is 5.0 cm and the total height is 50 cm ($H/D = 10$). The air is introduced through a fine bubble diffuser at the bottom of the reactor. Aeration flow rate was around 1.2 L/min, equivalent to a superficial air velocity of 0.6 cm/s. The reactor is equipped with a mechanical stirring system (100 rpm). The combination of air diffuser and agitator ensures the flow of air, liquid and solids mixing well in the reactor. The synthetic wastewater is stored in bottles at room temperature (20°C) during 2 days to avoid degradation of the compounds. The influent feeds the column from the bottom of the column. The experiment program was controlled automatically by the timers.

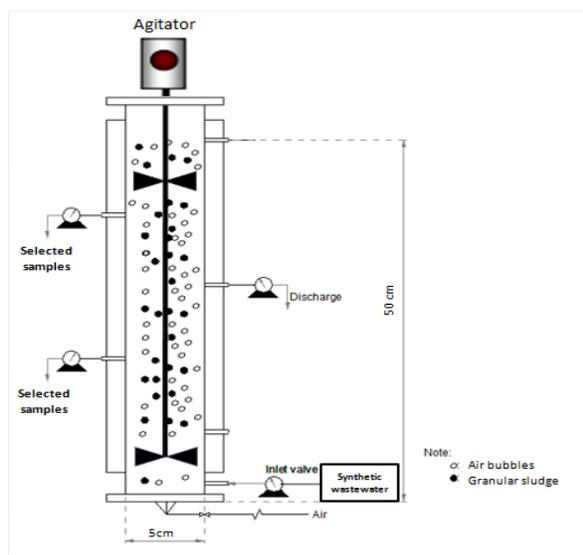


Figure 24: Scheme of lab-scale SBR

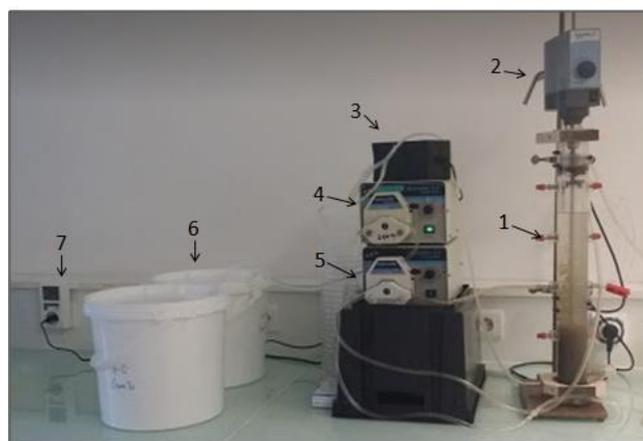


Figure 25: Photo of lab-scale SBR system:
1-Reactor; 2-Agitator; 3-Air diffuser; 4-Inlet pump; 5-Outlet pump; 6- Synthetic wastewater bottles; 7-timers

The system operate in sequential mode (Figure 26), with a cycle defined as follows: 2 min of feeding, 15 min of anoxic period with mechanical mixing, and aeration. The settling time was gradually decreased: 0-16th day: 15 min; 17-22nd day: 5 min; 22-25th day: 4 min and with 15 first minutes of mixing (air and agitation), 2 min of withdraw and 10 min of idling. 3 min of settling time were kept until the end of experiment. The total cycle time is 4h. The volume fraction of exchange is 50% and the hydraulic retention time (HRT) is 8h.



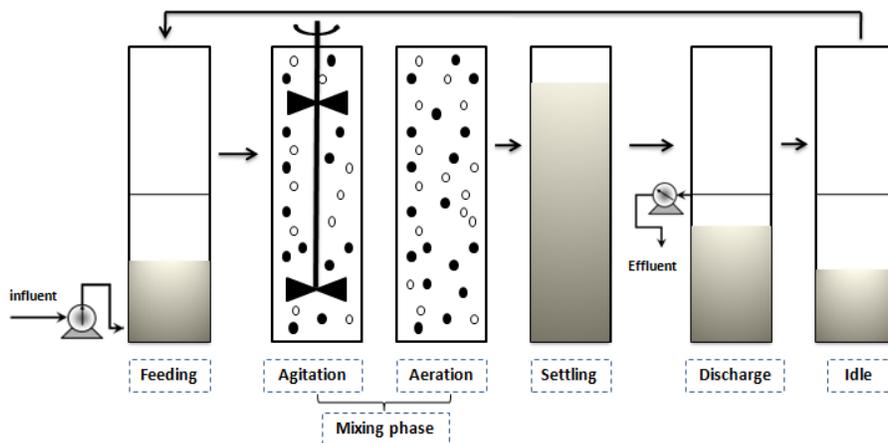


Figure 26: Description the SBR operating cycle.

- **Description of seed sludge and synthetic wastewater**

Seed sludge was taken from an aeration tank of the WWTP1. The seeding sludge had a fluffy, irregular, loose structure and brown color.

The composition of synthetic wastewater was selected to approximate a conventional organic effluent (which could be domestic effluent). The organic carbon source was supplied by using sodium acetate. The ratio of COD/NH₄⁺-N/PO₄³⁻-P was kept constant at 100/5/1. Nitrate is feeding in the reactor to maintain strictly anoxic conditions in the agitation-non-aerated phase (no anaeration). Table 11 summarizes the concentration ranges used in this study:

Table 11: Synthetic wastewater composition using for aerobic granulation

Compositions	Concentration (mg L ⁻¹)
CH ₃ COONa	1660
NH ₄ Cl	248
NaNO ₃	400
NaHCO ₃	358
K ₂ HPO ₄	73
MgSO ₄ · 7H ₂ O	25
CaCl ₂	30
FeSO ₄	20
Trace elements (H ₃ BO ₃ , ZnCl ₂ , CuCl ₂ , MnSO ₄ , etc.) were followed by Liu and Tay, (2007)	

After granulation (Appendices 1) the aerobic granular sludge was taken for Cu availability on sludge and sludge land spreading.



I.3. Summary of different sludge samples using in experimental study

Aerobic granular sludge from SBR lab-scale reactor was collected after 47 days of configuration. Their characteristics were analyzed and were compared with anaerobic granular sludge and conventional sludge. Due to a large number of sludge characterization and copper adsorption/desorption experiments, granular sludge and convention sludge were identified by different symbol. Table 12 lists the names of sludge using in this study.

Table 12: Summary of different sludge with their name and stabilization process

Sludge	Abbreviations	Kind of treatment	Origin	Characteristic of the installation
Conventional activated sludge	BE (WWTP 1)	Thickened sludge	Limoges WWTP 1	I.1.1; page 79
	BCh (WWTP 1)	Limed sludge	Made from BE WWTP 1 at lab	I.1.1; page 79
	BD (WWTP 1)	Digested sludge	Limoges WWTP 1	I.1.1; page 79
	BFP (WWTP 1)	Filter-press sludge	Limoges WWTP 1	I.1.1; page 79
	BCD (WWTP 1)	Centri-dried sludge	Limoges WWTP 1	I.1.1; page 79
	BC (WWTP 2)	Centrifuged sludge	Cahors (Lot) WWTP 2	I.1.2; page 80
	Cp1 (WWTP 2)	Composted sludge	Composting platform (without sludge from WWTP2)	I.1.2; page 80
	Cp2 (WWTP2)	Composted sludge	Composting platform 1 (with sludge from WWTP2)	I.1.2; page 80
	LR (WWTP 3)	Reed Bed sludge	La Bastide-Murat (Lot) (WWTP 3)	I.1.3; page 81
Granular sludge	BAN	Anaerobic granular sludge	UASB, Paper mill effluent	I.2.1; page 81
	BAE	Aerobic granular sludge	SBR lab-scale	I.2.1; page 81



II. Analytical methods

The various fractions of the sludge were characterized using several techniques to determine their biochemical composition and their structures and morphology. Table 13 provides a summary of the parameters determined by different analytical methods to characterize the sludge.

Granular and conventional sludge after stabilization process were prepared in two phases: soluble phase and particular phase. Soluble phase was created from centrifugation of total sludge at the condition of 6000 rpm, 40°C during 20 min, and then the supernatant was filtered by cellulose nitrate membrane 0.45 µm as soluble fraction. The pellet which was collected after centrifugation and filtration was used for particular phase.

The biological matrices of sludge were characterized by using several techniques to determine sludge characteristics. Firstly, their biochemical compositions such as protein, polysaccharides, humic like substances, etc. were analyzed. Secondly, the nature and quality of functional groups to the surface that and is responsible for interaction with micro-components (interactions or hydrophobic surface charges in particular) were determined by titration or IR spectra. Then heavy metal such as Cu content and distribution in sludge also were analyzed. A summary of the applied techniques is shown in Figure 27 and Table 13.

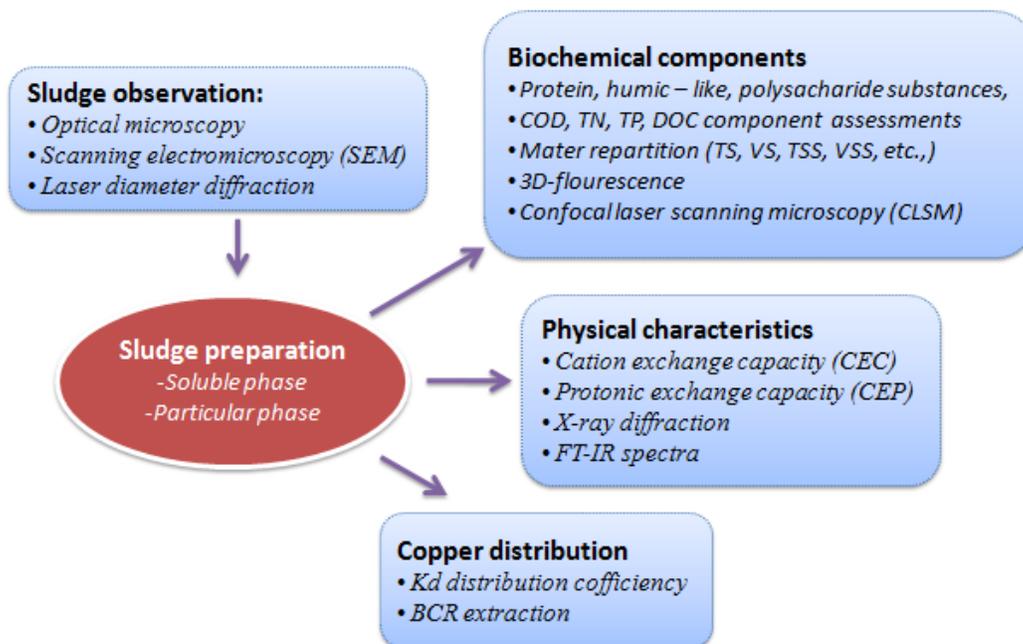


Figure 27: Summary of analyzes methods for sludge characteristic



Table 13: Summary of parameters determined by different analytical methods to characterize sludge

Fields	Parameter	Methods	Phase(s)	Separation	Objectives
Granular sludge observation	Light observation	Optical microscopy	total	-	Evaluate the granular size and the granular morphology
	Microbial composition	SEM	Particular	-	Evaluate the microbial distribution and granular structures
	Particular diameter distribution	LS TM 13 310	total	-	Evaluate the granular size and the changes in the specific surface
	EPS distribution	CLSM	particular	-	Evaluate the distribution of EPS components and granulation
Bio-chemical characterization	Quantity and distribution of material	TS, MM, VS, TSS, VSS, MMS (AFNOR 1997a)	Total Particular	Centrifugation, 6000g, 20 min, 4°C	Determination of sludge compositions
	Dissolved organic carbon (DOC)	COT-Nt meter SHIMADZU	Soluble	0.45 µm	
	chemical oxygen demand(COD); Total phosphorous (TP); Total nitrogen (TN)	Cuvette tests HACH LANGE	Total Soluble	0.45 µm	
	Protein and humic acid-like substances	(Lowry et al., 1951) modified by (Fr/olund et al., 1995)	Total Soluble	0.45 µm	
	Polysaccharides	(DuBois et al., 1956)	Total Soluble	0.45 µm	
	3D-fluorescence spectra	SHIMADZU RF-5301	Soluble	0.45 µm	
Physical characteristics of functional groups	Proton exchange capacity (PEC)	Protofit- titration acid/base	Soluble Particular	Centrifugation, 6000g, 20 min, 4°C	Determine the number of ionizable sites at surface of the sludge or in the soluble phase
	pKa and number of site in acid/base	Protofit- titration acid/base	Soluble Particular	Centrifugation, 6000g, 20 min, 4°C	The surface complexation model allows determine a number of pKa which may be treated as functional groups
	Cation exchange capacity (CEC)	(Hawari and Mulligan, 2006d)	Particular	Centrifugation, 6000g, 20 min, 4°C	Assess the ability of sludge bind cations by ion exchange
	Infrared spectra (FTIR)	FTIR Nicolet Magna 550	Particular	Centrifugation, 6000g, 20 min, 4°C	Assess the nature and quality development in functional groups
Behavior of Cu	Cu concentrations	Varian SpectraAA 800	Soluble Total	Centrifugation, 6000g, 20 min, 4°C	Evaluate the concentration distribution efficiency between soluble and total phase of Cu according to the treatment and
	Cu distributions	BCR extraction methods (Kazi et al., 2006)	Particular	Centrifugation, 6000g, 20 min, 4°C	Determine the Cu distribution in different fraction of sludge structures



II.1. Determination of material quantity and its distribution

The determination of the different fractions of the materials is carried out according to the standard method (AFNOR, 1997a).

II.1.1. Total solid (TS) and Volatile solid (VS)

To determine the total solids (TS) and volatile solid (VS) content, a known volume sludge (0.05 L) is introduced into a porcelain crucible. This crucible is placed in an oven (BINDER, Germany) at 105°C for 24 hours. Thus, water is evaporated and the dry matter is remained in the crucible. The porcelain crucible is weighed after cooling at room temperature in a desiccator (20-30 minutes) in order to prevent precipitation of humidity on samples or their supports. The difference in mass corresponds to the total solid contained in the sludge.

The crucible is then put in an oven (Nabertherm, Germany) at 550°C for two hours. After cooling, the crucible is weighed again, there remains only the mineral. The mass of volatile solid is obtained from the difference between the total solid weight and the weight of inorganic material.

The TS and VS values were calculation according to Equation 5 and Equation 6:

$$\text{Equation 5} \quad \text{TS} = \frac{m_{105^{\circ}\text{C}} - m_{\text{crucible}}}{v}$$

$$\text{Equation 6} \quad \text{VS} = \frac{m_{105^{\circ}\text{C}} - m_{550^{\circ}\text{C}}}{v}$$

Where: $m_{105^{\circ}\text{C}}$: is the weight of crucible (contained sludge) at 105°C

m_{crucible} : is the weight of initial crucible (without sludge) at 105°C

$m_{55^{\circ}\text{C}}$: is the weight of crucible (contained sludge) at 550°C

II.1.2. Total suspended solid (TSS) and Volatile suspended solids (VSS)

TSS and VSS are determined in the same way as TS and VS but on a base of centrifugation. The sample volume is then considered the volume of sludge prior centrifugation. The error in the measurements is in the order of 2%.

40 ml (0.04 L) of sludge was centrifuged in 20 minutes at 6000g and 4°C, then remove the supernatant and recover the pellet in the porcelain crucible which was weighed after washed and dried. This crucible was putted in the oven at 105°C for 24 hours. It was then weighed after cooling at room temperature during 20 – 30 minutes. The difference of mass will be showed for TSS value.



The crucible was placed continuously in the oven at 550°C in 2 hours and was taken the weight after cooling in desiccators. The VSS of sludge was calculated bases on the differences between the weight of crucible after 105°C and its weight after 550°C.

The TSS and VSS values were calculated according to Equation 7 and Equation 8.

Equation 7
$$\text{TSS} = \frac{m_{105^{\circ}\text{C}} - m_{\text{crucible}}}{V}$$

Equation 8
$$\text{VSS} = \frac{m_{105^{\circ}\text{C}} - m_{550^{\circ}\text{C}}}{V}$$

Where: $m_{105^{\circ}\text{C}}$: is the weight of crucible (contained particular sludge) at 105°C

m_{crucible} : is the weight of initial crucible (without particular sludge) at 105°C

$m_{55^{\circ}\text{C}}$: is the weight of crucible (contained particular sludge) at 550°C

II.2. Sludge chemical and biochemical characteristics

II.2.1. Determination of Oxygen chemical demand (COD), nitrogen and phosphorus measurement

Chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP), PO_4^{3-} - P (orthophosphate) were carried out in triplicate according to HACH kits reagent sets and their colorimetric measurement was determined by HACH-DR/2010 spectrophotometer device (Table 14). Sludge samples were divided into two phase, soluble and total phase. The soluble phase was obtained after centrifugation of total sludge after 20 min at 6000g and 4°C and then filtration of supernatant through the cellulose nitrate membrane 0.45 μm . There are different measuring ranges for each measurement according to sample concentration. Sample was diluted with distilled water if it was necessary. The measurements are performed in triplicate. The standard deviation of the measurements is in the order of 20% for total sludge and 10% for soluble fraction.

Table 14: Parameter and analytic methods

Parameters	Range of concentration	Method
COD	0 – 150 mgO ₂ /L	LCK 500 HACH – DR 2010
	100 – 2000 mgO ₂ /L	LCK 541 HACH – DR 2010
TP and PO ₄ ³⁻ -P	0,05 – 1.5 mg PO ₄ ³⁻ -P/L	LCK 349 HACH – DR 2010
	2.0 – 20 mg PO ₄ ³⁻ -P/L	LCK 350 HACH – DR 2010
TN	20 – 100 mg/L	LCK 338 HACH – DR 2010



II.2.2. Proteins and humic – like substances

Protein content in sludge is performed according to the colorimetric method of (Lowry et al., 1951) and the modification of (Frolund et al., 1995). This colorimetric assay is based on two reactions: 1) the Biuret reaction: based on the reduction of Cu^{2+} which then binds to protein forming a Cu^+ peptide complex with biuret ($\text{NH}_2\text{-CO-NH-CO-NH}_2$) in alkaline medium, and 2) subsequent reduction of the Folin-Ciocalter reagent by this complex based on the reaction between phosphomolybdate and phosphotungstate with tyrosine and tryptophan to give a blue coloring.

Furthermore, a modification to the Lowry assay employed by (Frolund et al., 1995) claimed to enable separation of absorbance due to protein and that from the humic fraction, by inclusion and exclusion of copper sulphate from the Lowry reagent. The color is measured with and without the addition of CuSO_4 in the analysis. When CuSO_4 is omitted, coloring is due to humic compounds and chromogenic amino acids. Without CuSO_4 , color was developed by BSA (Bovine Serum Albumin) was reduced by 20% but not for humic – like substances. The absorbance respectively due to proteins and humic-like acid substances are then calculated with the following formulas (Frolund et al., 1995):

$$A_{\text{total}} = A_{\text{protein}} + A_{\text{humic}}$$

$$A_{\text{blanc}} = 0,2A_{\text{protein}} + A_{\text{humic}}$$

$$A_{\text{protein}} = 1,25 (A_{\text{total}} - A_{\text{blanc}})$$

$$A_{\text{humic}} = A_{\text{blanc}} - 0,2A_{\text{protein}}$$

With:

A_{total} = the total absorbance with CuSO_4

A_{blanc} = the total absorbance without CuSO_4

A_{humic} = the absorbance due to humic compounds

A_{protein} = the absorbance due to proteins

The concentrations of protein and humic – like substances are calculated based on line calibrations which were established by Bovine Serum Albumins (BSA, Sigma A7906, 98 %) and humic acid (Fluka 53680) standard solutions, respectively. The concentration ranges extend from 0 to 200 mg/L BSA and Humic acids.

The protocol is shown in Table 15. The reagents and the solutions are introduced in the test tubes. The standard deviation of protein in triplicate measurements is ± 70 mgBSA/L ($\pm 5\%$) on the total fraction and ± 8 mg BSA/L (1 to 7 %) in the soluble fraction. For the Humic – like substances, the standard deviation values are ± 16 mgAH/L ($\pm 4\%$) and ± 1 mgAH/L (1 to 13 %) in the total and soluble fractions respectively.



Table 15: Protocol for determination of Proteins and Humic – like substances

Tubes	with CuSO ₄	without CuSO ₄
Solution C1 (ml)	5	0
Solution C2 (ml)	0	5
Volume of sample solution (ml)	1	1
Shake the tubes by using a vortex		
Keep the tubes standing in 10 minutes		
Solution D	0.5	0.5
Shake the tubes by using a vortex		
Keep the tubes standing in 30 minutes in the dark		
The absorbance (Abs) value were analyzed by using the spectrophotometer device at the wavelength of 750 nm		

With: Solution A: 500 ml A1 + 500 ml A2

- **A1:** Na₂CO₃ 4% m/V, this solution was prepared by dissolved 53.96g Na₂CO₃.10H₂O or 20g Na₂CO₃ in 500 ml distilled water.
- **A2:** NaOH 0,2 M: diluting 100 ml of NaOH 1M in 500 ml distilled water

B1: CuSO₄.H₂O at 1% m/V: Dissolving 6.39g CuSO₄ anhydride in 1 L distilled water

B2: K and Na tartrate at 2% m/V: Dissolving 2g of tartrate in 100 ml distilled water

C1: 98 ml A + 1 ml B1 + 1 ml B2

C2: 98 ml A + 1 ml B1 + 1 ml B2

D: Folin reagent 1N: Dilute 2 times the commercial reagent solution 2N

The use of the term “humic – like substances” is a little unfair since it does not act exclusively of substances from the ground, but is commonly used in the literature concerning sludge and extracellular polymers. Thus, we use the specific phrases “Humic – like substances” in the protocol which is characterized by (Frolund et al., 1995).

II.2.3. Polysaccharide

Colorimetric method is the most widely assay to date for determination of polysaccharides concentration in aqueous solution (DuBois et al., 1956). The basic principle of this method is that carbohydrates, when dehydrated by reaction with concentrated sulfuric acid, produce furfural (a heterocyclic aldehyde) derivatives. Further reaction between furfural derivatives and phenol develops detectible orange color. The standard procedure of this method is as follows: a 1 ml aliquot of a carbohydrate solution is mixed



with 1 ml of 5% aqueous solution of phenol in a test tube. Subsequently, 5 ml of concentrated sulfuric acid is added rapidly to the mixture. After allowing the test tubes to stand for 10 min, they are stirred by vortex for 30s and placed for 20 min in a water bath at 25°C for color development. Then, light absorption at 490 nm is recorded on a spectrophotometer. Reference solutions are prepared in identical manner as above, except that the 1 ml aliquot of carbohydrate is replaced by distilled water. The phenol used in this procedure was redistilled and 5% phenol in water (w/w) was prepared immediately before the measurement. The concentration of polysaccharides is then calculated based on calibration curve (range of 0 to 100 mg glucose/L). Polysaccharide standard solution was prepared by glucose standard solution (D⁺ glucose, VWR Prolabo RECTAPUR 24379-363). The measurements are performed in triplicate. The standard deviation of the measurements is in the order of $\pm 20\%$ on the total fraction and between 1 and 10 % for soluble fraction.

II.2.4. Lipid

The colorimetric sulfo-phosphor-vanillin (SPV) method developed by Chabrol et al (1937) is an attractive method for lipid measurement. The SPV method has been modified for the diverse application such as in case of sludge. Billa et al., (2014) have recently modified the method for use with small sized samples. For principal, the SPV reaction is performed in two steps, initial reaction of the lipids with concentrated (typically $\geq 95\%$) sulfuric acid at high temperature followed by a second reaction of the derived products with vanillin in the presence of phosphoric acid. Consensus understanding is that a positive SPV reaction requires the presence of double bonds or free hydroxyl groups within the lipid analysis. The chemical reactions are complex and are through to involve formation of relatively stable carbonium ion in the initial reaction followed by generation of a pink chromophore upon addition of vanillin to the reaction.

- **Reagent preparation of analysis**

- The phosphor vanillin reagent was prepared by placing 3.0 g of vanillin into 0.5 L hot water. After dissolution of the vanillin, the vanillin solution was added to 2 L of 85% phosphoric acid. The reagent was stored at room temperature in darkness in an amber color bottle.
- Oil standard solution 1 g/L (soybean oil or olive oil or commercial oil) was prepared by adding 50 mg of neat soybean oil to a 50 ml volumetric flask and diluted to volume using methanol/chloroform (1:1: v/v)
- The methanol/chloroform (1:1: v/v) was also prepared in the dark bottle

- **Lipid analysis method**

With the oil standard solution, a standard curve was prepared by pipetting 50, 100, 200, 300, 400, 600 μ l of oil standard solution into glass tubes of 10 ml, respectively. For each tubes, 500 μ l of the chloroform: methanol extract was transferred into a 13 mm culture tube. All the tubes (without the caps) were then placed on the



heater at 100°C (temperature range 100°C – 110°C) for 30 min to evaporate all solvent from the tubes. The tubes were allowed to cool to room temperature, and 2.5 ml of concentrated sulfuric acid was added, closed strongly the caps for all culture tubes, and then putted them in the heater for 20 min at 100°C. Maintaining the temperature above 100°C is crucial in this step for the hydrolysis of lipid esters. The tubes were then allowed to cool to room temperature and 5 ml of phosphor-vanillin reagent was added to each tube while mixing by vortex. After a minimum of 30 min for color development, the absorbance at 525 nm was measured on UV-Vis spectrophotometer DR/4000U-Hach.

For the sludge samples, 5 ml of sludge were mixed with 5 ml of methanol and chloroform (1/2:v/v) in 5 min, then centrifuged in 20 min, 6000g at 4°C. The supernatant from the extract was removed in other glass tube as lipid extract solution. Then colorimetric steps were applied for extracted solution as standard solution. Then, amounts of lipid in extract were computed bases on standard curve. Lipid extract solution of sludge was replied two or three times.

Depending on the concentration of lipid in each kind of sludge, the volume of sludge solution for lipid extraction is changed.

II.2.5. Dissolved organic carbon (DOC)

The measurement of the dissolved organic carbon is performed using a Shimadu TOC meter TOC – L (The accuracy is $\pm 2\%$ and the detection limit is 50 $\mu\text{gC/L}$ with calibration range of 0 – 50 mgDOC/L which were prepared from potassium biphthalate standard solution of 1 gDOC/L). The measurement is carried out after acidification with HCl 1M and bubbling synthetic air quality during 3 min, to degas the inorganic carbon. The sample is then injected into an oven at 720°C and CO₂ produced by the combustion of the organic material is then quantified by IR measurement. These measurements are carried out in duplicated.

II.2.6. Spectroscopy de fluorescence –three dimension (3D)

The analysis is performed at room temperature for sludge soluble fraction study. Some samples highly concentrated DOC had to be diluted in distilled water to obtain an unsaturated florescence spectrum. The apparatus used was a 3D spectrofluorometric Shimadzu-RF-5301 PC with acquisition software “Panorama Fluorescence 2.1”, and provided with an optical path of 1 cm quartz cuvette. The unit stability is checked prior each series of analysis using the fluorescence due to the Raman scattering of ultra-pure water. The change in fluorescence intensity measured at $\lambda_{\text{ex}} = 350 \text{ nm}$ and $\lambda_{\text{em}} = 395 \text{ nm}$ is below 2%. Excitation-emission matrices are plotted for excitation wavelengths ranging from 220 nm to 450 nm in steps of 5 nm and the emission wavelength is between 250 nm and 550 nm, in step of 1 nm.

Chen et al., (2003) defined in their work to map areas with different lie organic compounds such as protein-like and humic-like substances and their excitation/emission wavelengths were present in Table 16 and Figure 28.



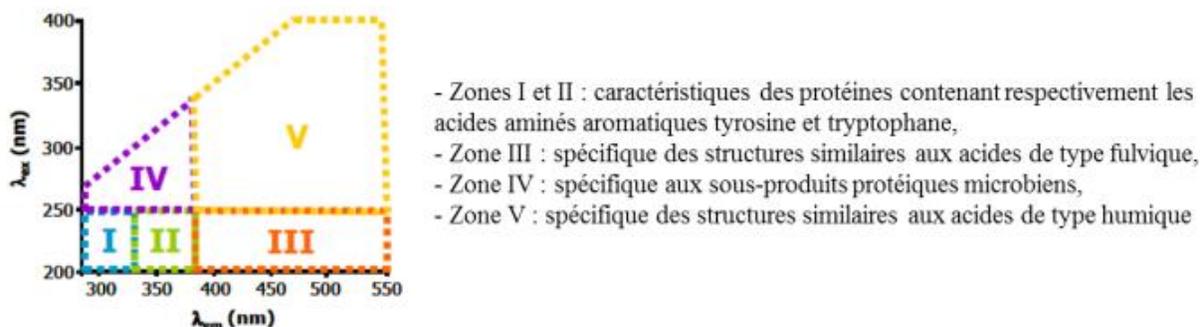


Figure 28: Mapping different areas of fluorophores (Chen et al., 2003; Barret, 2009))

Table 16: Different excitation/emission wavelength correspond with region of components

Main components	Zone	Excitation wave length (nm)	Emission wave length (nm)	Components
Protein like substances	I	200 – 250	250 – 300	Tyrosine
	II	200 – 250	300 – 380	Tryptophan
	IV	> 250	250 – 380	Sub-microbial protein products
Humic – like substances	III	200 – 250	> 380	Fulvic acid (FA)
	V	> 250	> 380	Humic acid (HA)

II.2.7. Confocal laser scanning microscopy (CLSM)

Chen et al., (2007) was the first to describe the distribution of protein, α - and β -polysaccharides and lipids in aerobic granular sludge using a novel staining scheme and confocal laser scanning microscopy (CLSM). This work selectively hydrolyzed lipids, proteins and α - and β -polysaccharides using specific enzymes. Briefly, fluorescein isothiocyanate (FITC) was utilized to stain amine reactive compounds such as proteins and amino sugars. Fluorescently labelled lectin concanavalin A (Con A) conjugated with tetramethyl rhodamine was applied to bind the α -glucopyranosyl sugar residues. Nile red was utilized to stain lipid. All the dyes and target staining was showed in the Table 17.

Table 17: Dyes and target staining by (Chen et al., 2007)

Dye	Excitation (nm)	Emission (nm)	Targets
ConcanavalinA conjugates (Con A)	543	550-600	Polysaccharides
Fluorescein isothiocyanate (FITC)	488	500-550	Proteins
Nile red	515	625-700	Lipids



Prepare standard staining and buffer solutions

• **Standard staining solution**

- Nile red: 0.02 mg powder/ 2 ml of acetone to obtain 10 mg/L Nile red solution
- FITC: 0.02 g/2 ml of distilled water for 10 g/L FITC solution
- ConA: 0.5 mg/ 2 ml of distilled water to get 250 mg/L ConA solution

All solutions are stored at -4°C

• **Buffer solution**

- Sodium bicarbonate buffer 0.1 M (pH = 9): Prepare separately a solution of anhydrous sodium carbonate 0.2 M (2.2 g/100 ml of distilled water) and a solution of sodium bicarbonate 0.2 M (1.68 g/100 ml of distilled water). The sodium bicarbonate was created by combining: 4 ml of carbonate solution and 46 ml of bicarbonate solution in distilled water and for total volume of 200 mL. The pH of solution was controlled by NaOH 0.1 M or HCl 0.1 M with the pH probe.
- Phosphor saline buffer – PBS 0.1 M (pH = 7.4): The mixed of powder contents: NaCl (8.0 g); KCl (0.2 g); Na₂HPO₄ (1.44 g); KH₂PO₄ (0.24 g) was dissolved in 1.0 L of distilled water and adjusted the pH to 7.4 by HCl solution and pH device.

Staining process:

One kind of sludge was prepared in 2 samples: 1 sample was stained with FITC and ConA, the other one was stained with FITC and Nile red, due to Con A and Nile red has closely the wavelength of emission and excitation.

The first sample: 0.1 M Sodium bicarbonate buffer (100 µl) was added to maintain the amine group in non-protonated form that was followed by a 50 µL FITC solution and mixture stirred at room temperature in 1 hour and in the dark place. Continuously, 100 µl of the Con A was incubated with the sample for another 30 mins in the dark.

The second sample: the process will be repeated with FITC and then 100 µl of the Nile red was incubated with the sample for 10 min in the dark (because the acetone evaporate very quickly so the staining will be very fast).

Before staining, 2 ml of sample was added in centrifuged tube and centrifugation at 6000g during 10 min and 4°C. Then, the supernatant was removed but the pellets were always kept fully hydrated during staining. Between each staining, the stained sample was washed twice (or more than twice) with phosphate buffer saline (pH = 7.4) to remove extra stain and the stained sample were stored at 4°C.



A drop of the stained suspension was deposited on a glass slide, covered with a cover slip, and sealed with a clear varnish to prevent drying of the sample during visualization with the microscopes. It should be remembered that using of the cover slip the original shape of the aggregates in the activated sludge will be deformed. The observation under microscopic need to be fast because the stained sample will dry quickly, it affects to the quality spectrum when you analyze with the Velocity software.

After staining, the stained sample was observed under CLSM- Zeiss 510 META, Germany.

II.3. Determination of functional groups distribution on sludge

II.3.1. Determination of sludge's surface properties – Acid-base titration

Acid-base titration is used to characterize the chemical functions in the soluble and particulate fractions of the sludge in order to observe the changes in potential functional groups present. The aim is to determine the acidity constant (pKa) and the corresponding number of locations, both on the surface and the floc in the soluble fraction. The total proton acceptor site that is to say of the ionizable groups is called proton exchange capacity (CEP).

The protocol described below is from the work of Lachassagne (2014) and Laurent (2009).

II.3.1.1 Total and soluble fraction preparation

Soluble fraction: 2 x 40 ml of sludge is centrifuged. The supernatant is collected for soluble phase titration.

Total fraction: The pellets in the centrifuge tubes after remove soluble phase are re-suspended in 40 ml of NaNO₃ 0.01 M and centrifuged again. The supernatant was discarded and the procedure repeated once again. After the final rinse, the pellet is a last re-diluted in NaNO₃ solution for a final volume 2 x 50 ml. This suspension was then used for titration of the biomass.

- **Total fraction titration**

The biomass suspension (50 ml) is transferred into a thermostatic cell (25°C). This cell is covered by Parafilm. The cover is pierced to let the pH probe, the injection capillary and a hose for nitrogen bubbling pipe. The suspension is stirred using a magnetic stirrer. Before any measurement, the system is degassed for 30 min in nitrogen to eliminate CO₂ atmosphere. A positive pressure of nitrogen is then maintained during measurement.

Titration are performed in a pH range from 2.5 to 10 at 25 ± 1°C by using a 716 NET Titrino automatic titration (Metrohm Ltd). For limed sludge, the pH is being higher; the titration process was programmed to pH 12. Before each experiment, the pH electrode is calibrated. The titration is programmed dynamically (DET) for installed method. This mode adds varying amounts of solution based on pH changes: smaller additions are made when the pH changes are greater and the drift signal is 6 mV/min. Two separate interchangeable burettes 20 ml are used: one for the acid (nitric acid) and one for the base (sodium hydroxide).



In general, the protocol is adapted to the type of sludge as regards volumes and the concentrations of acid and sodium hydroxide added. Concentrations of acid or base are 0.01; 0.1; 1.0 mol/L. These solutions are prepared extemporaneously from commercial solutions. For titration of 2 x 50 ml total fractions, the first is titrated to pH 2.5 by nitric acid 0.01 mol/L (for example). The data is saved and changed burette. The second sample is then titrated by sodium hydroxide 0.01 mol/L (for example) up to pH 10.

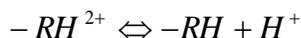
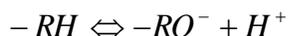
- **Soluble fraction titration**

50 ml of the supernatant sludge which was collected after first centrifugation is transferred to the titration vessel. The protocol is the same one used for the biomass fraction except that the titration with the acid and the base are performed on the same solution: the first step is titrated to pH 2.5 with nitric acid 0.01 mol/L (for example) and then to pH 10 with sodium hydroxide 0.01 mol/L (for example).

II.3.1.2 Data analysis

The data of titration are recorded on a PC using the software Tinet (Metrohm). Titration curves are then interpreted by a non-electrostatic adsorption model protons using software PROTOFIT. PROTOFIT is a tool for analyzing the data after acid-base titration. It is primarily designed for optimization of protonation models of different types of mineral or organic origin surfaces. PROTOFIT can optimize acidity constants from a pattern selected from four available, this kind for a four surface locations. These models include the model of the double layer (DLM: Double layer model), Constant capacitance model (CCM), Donnan Shell model (DSM), and a non-electrostatic model (NEM). The activity coefficients can be considered as equal to 1. For a detailed presentation of surface protonation of the modeling and the operation of the software PROTOFIT are available in the work of Turner and Fein (Fein and Turner, 2006).

The adsorption model of non-electrostatic protons chosen based on surface reactions forming positive or negative monovalent groups as equation:



In these equations, R represents an organic functional group.

The model is optimized based on the assumption that the pH is fixed at each stage of the titration and the surface is in equilibrium with the solution. A number of exchange reactions of protons, the shape of the above equation assumed to take place at the surface, each equation corresponds with the equilibrium constant and a number of sites. The law of mass action is expressed in the non-electrostatic model according to the following equations:



$$\frac{[-RO^-][H^+]}{[-RH]} = K_1$$

$$\frac{[-RH][H^+]}{-RH^{2+}} = K_2$$

The choice of the number of types of sites and their properties (acid, base, amphoteric) is performed by configuration offering the best fit between experiment data and calculated with the model (Laurent et al, 2009).

The model provides the corresponding concentration for each pKa, which corresponds to the PEC in the sites unit of mol/kgTS.

II.3.2. Cation exchange capacity

The CEC is determined according to the protocol used by Hawari and Mulligan (2006d) on a sludge sample concentrated by centrifugation.

The capacity of the biomass to bind cations by ion exchange mechanism is proportional the number of negative charges per unit weight of biomass. The capacity of the biomass retained cations, i.e. the number of negatively charged sites per unit mass of biomass can be expressed quantitatively by a parameter called the Exchange Capacity Cations (CEC). There are many ways of measuring the CEC. Most are based on principle of cation displacement, that is to say, a method in which cations occupying the exchange site are moved “rinsing” biomass with excess cations from a reagent, such as ammonium acetate (NH_4^+ is the cation “moving”) (Hawari and Mulligan, 2006d).

The protocol used is derived from that used in their work on granular sludge previously dried by Hawari and Mulligan, (2006d, 2006e). In this study, the sludge will not be dried before handing but concentrated by centrifugation so as to maintain the surface properties of fresh slurry. This protocol is described in different steps:

Step 1: 40 ml of a liquid sludge was transferred to the centrifuge tube then centrifuged at 6000g in 20 min and 4°C. The supernatant was removed and the pellet was kept in the tube for the next steps.

Steps 2: 20 ml of potassium acetate 1M was added in the centrifuge tube which contents only pellet after step 1. This tube was agitated at 180 rpm in 30 min. Then it was centrifuged at 6000g in 20 min and 4°C. The pellet on the tube was separated after removed supernatant. This step will be replied 2 times.

Step 3: this step is similar step 2, the difference is: 20 ml of potassium acetate 1M was replaced by 20 ml of distilled water.

Step 4- final step: 25 ml of ammonium acetate 1M was added in the centrifuge tube after step 3. All the conditions of agitation and centrifugation in the step 3 were reminded in step 4. This step was repeated 2 times. After centrifugation, the first and second supernatants were kept separately and then filtrated by cellulose



nitrate membrane 0.45 µm and stored in fridge at 4°C until analysis. Before analyzing potassium concentration by atomic adsorption device, the filtrated solutions were diluted 250 times with distilled water.

Note: In the step 1, the volume of sludge was taken depending on the concentration of sludge sample.

The potassium concentration in the supernatant is measured by atomic emission in the Flame retardant according to the standard (diluted to reach a concentration of < 2 mg/L is about 250 times) (AFNOR, 1997b). The potassium concentration thus determined (expressed in meq/100 g) is equal to the CEC (Equation 9).

Equation 9
$$\text{CEC} = \frac{x \cdot D \cdot V_{\text{sample}} \cdot 100}{\text{VSS} \cdot 9.38352}$$

Where: **CEC**: is cation exchange capacity (meq/100g MVS)

x: is Potassium concentration in the diluted sample assayed by flame (mg/L)

D: is dilution factor used for the determination flame

V_{sample}: is final volume of the sample (50.10⁻³ L)

VSS: is volatile suspended solid concentration of studied sludge (g/L)

II.3.3. Infrared spectra

The infrared spectroscopy is used to study the structure of the organic material or mineral. The mid-infrared regards wavelength radiation ranging from 4000 to 400 cm⁻¹. An IR spectrum is made up of absorption bands corresponding to different modes of vibration of the molecule. Obtaining an IR spectrum requires analysis of all wavelengths emitted by the source, after absorption by the sample. Each absorption band can be attributed to a characteristic group.

The spectra was produced from sludge samples having undergone preparation follows: a volume of 40 ml of slurry after treatment 3 times is centrifuged (6000g, 20 minutes, 4°C) and the pellets were suspended in distilled water. The pellets obtained after the third centrifugation were then recovered in crucible porcelain and dried for at least 24 hours at 105°C. The obtained dried sludge was finely ground in an agate mortar. A pellet of 13 mm in diameter is then manufactured through a mold adapted by mixing about 5 mg of the powder thus obtained with 180 mg of KBr. This mixture was then pressed at a pressure about 3 t/cm² to obtain the patch. White is performed on a pellet containing only KBr. The spectra are finally registered (400 to 4000 cm⁻¹) with a Nicolet Magna 550 FTIR spectrometer and operated using the OMNIC software.

II.4. Sludge observation

II.4.1. Optical microscopy

To visually identify the structure of aggregates, microscopic observation of sludge was carried out using an optical microscope Zeiss Stemi SV6 (objective x 0.8; 1.0; 1.2; 1.6; 2.0; 2.5; 3.2; 4.0; 5.0). The



photographs are taken with the LD camera (Microvision Instrument, Germany). Using a dissecting microscopy (Loup SV6) is preferred to characterize aggregates when they reached a large size that can no longer see them in full light microscopy. The insertion of the ladder on the photo microscope or magnifying glass and the image processing are done using the computer program Videomet.

II.4.2. Scanning electron microscopy (SEM)

This method is used for observation of microbial composition distribution and the shape of aggregation which cannot obtain by optical light microscope. Sample for SEM were washed with phosphate –buffered saline (PBS) and fixed in 2.5% glutaraldehyde overnight at 4°C. Fixed aggregates were washed with 0.10 M sodium cacodylate buffer; dehydrated by successive passages through 25, 50, 75, 80, 90, 95, and 100% ethanol, then sludge were dried in freeze at 4°C (Weber et al., 2007). Finally, the completed sludge forming process was investigated by scanning electron microscope (SEM) TM 3000 (Hitachi Instrument, Japan).

II.4.3. Laser diameter diffraction

Measuring the granular size is determined using a LS (TM) 13 320 particle size analyzer (Beckman Coulter Instrument, American). This technique relies on the property of materials to diffract light. Any particle illuminated by a laser beam diffracts light in all directions with an intensity distribution that depends on its size.

Depending on the properties of the material, the measurement range varies between 0.02 and 2000 µm. The particle size of sludge yielded aggregate size distributions and thus access to the most likely diameter of the aggregates (granule and floc) in terms of volume percentage. The example of the volume distribution of the sludge was shown in Figure 29.

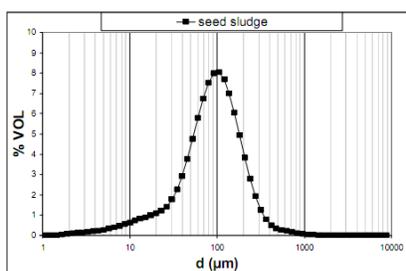


Figure 29: The aggregate distribution of sludge following the volume distribution

It can be noticed that the interpretation of the results must be carry out with the caution of diffraction phenomenon and methods which are not easily applicable in certain circumstances, for example:

- The particle are spherical, homogeneous and isotropic
- The particle movement are random
- The particle are sufficiently distant from each other
- The medium is transparent and does not absorb laser beam.



II.5. Copper determination in sludge

II.5.1. Sample preparations: mineralization process

- **Soluble fraction**

The samples are filtered on a cellulose nitrate membrane 0.45 μm . They are then acidified with a few drops of concentrated nitric acid (69%) and stored in tubes polyethylene at 4°C.

- **Total fraction**

Total fraction is prepared by mineralization method using Multivalve GO (Microwave digestion system with DMC directed multimode cavity – Anton Paar): 5 ml of sludge are stayed in the Teflon tube of device. Some solutions was continuously added in the tube: 3.0 ml of H_2O_2 30% solution were putted in the tube at the beginning, mixed softly and kept the tube quite during 30 min; 3.0 ml of concentrate nitric acid was added later and stored culture tube at room temperature during 1 hour; 6.0 ml of concentrate chloride acid was manned at the end of process. The tube was kept during one night before transferred to microwave device and setup and check all the conditions for the automatic mineralization program on the device before starting. When the process finish and the samples cooled at room temperature, they are filtered by cellulose nitrate membrane 0.45 μm and stored at 4°C for further study.

Depending on the concentration of metal elements to be measured, the measurements are carried out on a flame or furnace atomic absorption spectrometer.

II.5.2. Flame - Atomic absorption Spectroscopy (AAS)

Copper concentration was measured using a Varian spectrometer 880Z. The analysis is based on the absorption of photons by the atoms in the ground state at a specific wavelength of the element to be assayed. The sample in the liquid phase is sucked and injected in a flame air/acetylene which will ensure the atomization of the element, the absorbance is then measured and the concentration of the element is determined by a calibration curve which formed in the measurement range for each element (from 0 to 5 mg/L for Copper determination). The samples were diluted with distilled water if necessary.

- **Furnace - atomic absorption spectrometry (F-AAS)**

The apparatus used is an absorption spectrometer equipped with electrothermal atomization with a background correction system Zeelan marque Varian SpectrAA 800. The measurement principle is the same as flam except that the atomization of the element is ensured within an electrothermal graphic furnace which increases the sensitivity. In order to avoid interference phenomena due to the complex matrices studied, element analysis is performed by the addition method after appropriate sample dilution method. For each



sample, known concentration and increasing of the element to be assayed are added before the measure. The line obtained is used to calculate the initial concentration of individual trace element concerned.

II.5.3. Copper distribution determination: BCR extraction

The conventional BCR protocol (the community Bureau of Reference now the European Union “Measurement and Testing Programmer”) often requires to extremely timing consuming (48h). Recently, The BCR extraction method was modified by the use of compromise sonication conditions in ultrasonic bath. Following this method, the sequential extraction procedure could be completed in around 30 min. In this work, copper was extracted in different fractions of sludge, soil and sludge amended soil by using modified BCR extraction method which were described in study of (Kazi et al., 2006).

➤ BCR materials

Soil or sludge or sludge amended soil samples were dried at 105°C after 24 hours and sieved through a 200 µm mesh screen (after removing stones and other debris) and stored in polyethylene bottles until further use.

Deionized water was used for all dilutions. All the standards, reagent solution and samples were kept in polyethylene containers. Acetic acid (glacial, 100% - Fisher Scientific, Loughborough, Leicestershire, UK), Hydroxyl ammonium chloride (ACROS organics, New Jersey, USA), hydrogen peroxide (30% - Fisher Scientific, Loughborough, Leicestershire, UK), and ammonium acetate and HNO₃ (65%, Suprapur Merck, Darmstadt, Germany) were supper pure quality. All the plastic and glassware were cleaned up by soaking in dilute HNO₃ 0.1N and were rinsed with deionized water prior to use.

Solution I (acetic acid, 0.11 M): Distilled glacial acetic acid, 6.29 ± 0.2 ml, was added (in a fume cupboard), to about 500 ml of deionized water in a 1000 ml polyethylene volumetric flask and made up to exactly 1000 ml to obtained an acetic acid concentration of 0.11 M.

Solution II (hydroxyl ammonium chloride, 0.1 M, pH = 2.0): Hydroxyl ammonium chloride (6.95 g) was dissolved in 900 ml of deionized water. The solution was acidified with concentrated nitric acid to pH 2 and made up to 1000 ml. This solution was prepared on the day the extraction was carried out.

Solution III (ammonium acetate, 1.0 M) ammonium acetate (77.08 g) was dissolved in 900 ml of deionized water. The solution was acidified to pH 2.0 with concentrated nitric acid and made up to 1000 ml.

Note: All the BCR material amounts were calculated bases on the amount of sludge sample analysis; they were used immediately after preparation.



➤ **The BCR sequential extraction procedure**

Step 1: Exchangeable/acid soluble fraction (F1)

20 ml of acetic acid (0.11 M) was added to 0.5 g sludge in a 50 ml centrifuge tube with stopper and extracted by sonication in 7 min at 20 W and room temperature. The extract was separated from the solid residue by centrifugation at 6000g for 20 min and 4°C and supernatant liquid was decanted and filtrated by cellulose nitrate membrane 0.45 µm into polyethylene container and frozen until analysis.

Step 2: Reducible fraction (F2)

20 ml of a freshly prepared of hydroxylamine hydrochloride (0.1 M) was added to the residue from step 1 in the centrifuge tube, re-suspended by manual shaking, and then extracted by sonication in 7 min at 20 W and room temperature. The extract was separated from the solid residue by decantation, centrifugation, and filtration as in step 1. The extract was retained in a polyethylene container, as before, for analysis.

Step 3: Oxidation fraction (F3)

10 ml of hydrogen peroxide (30%) was added carefully to the residue in centrifuge tube and was digested by sonication in 2 min at 20 W and room temperature. A further aliquot of 25 ml of ammonium acetate (1.0 M) was added to the moist residue and sonicated in 6 min at 20 W and room temperature. The extract was separated from the solid residue by decantation, centrifugation, and filtration and storage as in step 1 and step 2. The solid residue was stored in the oven at 40°C.

Step 4: Residual fraction (F4)

The residual fraction was digested using the aqua regia HCl/HNO₃ (1/2: v/v) solution. This step was mannered similar total metal digestion analysis.

Two independent replicates were performed in parallel for each sample. And blanks were measured for each set of analyses, respectively. An internal check was performed on the sequential extraction results by comparing the total amounts of metals extracted by different reagents during the sequential extraction procedure with the results obtained by total digestion. All the extraction steps were presented in Figure 30.



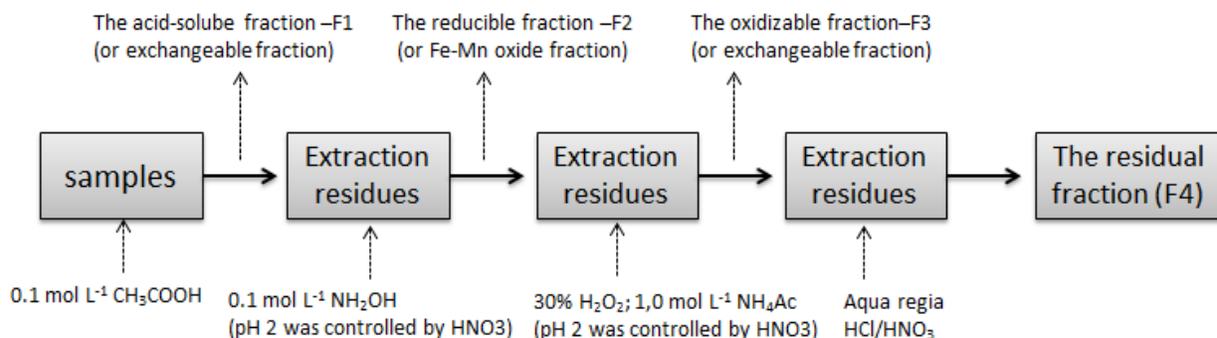


Figure 30: BCR sequential extraction method scheme

➤ **Result calculations**

The Cu concentration in the different fractions was calculated following the Equation 10:

$$\text{Equation 10} \quad m_F = \frac{C.V}{10^6.m}$$

Where: m_F : is Copper concentration in each fraction (mg Cu/g)

C : Copper concentration in each extraction solution according to different fractions was determined by F-AAS ($\mu\text{g L}^{-1}$)

V : Volume of extraction solution (ml)

m : the weight of sample (sludge or soils or sludge amended soil samples) (g)

III. Experimental protocols for studying the behavior of Cu in sludge and sludge amended soil

The objective of this part is to determine the potential Cu adsorption/desorption on granular (anaerobic/aerobic) and conventional sludge and soil. The Cu leaching availability when sludge amended on soils (grass land and paddy land), was established on batch and column tests. After Cu leaching process, Cu distribution in different fraction of sludge and sludge amended soil was investigated. Summary of the study was presented in Figure 31.



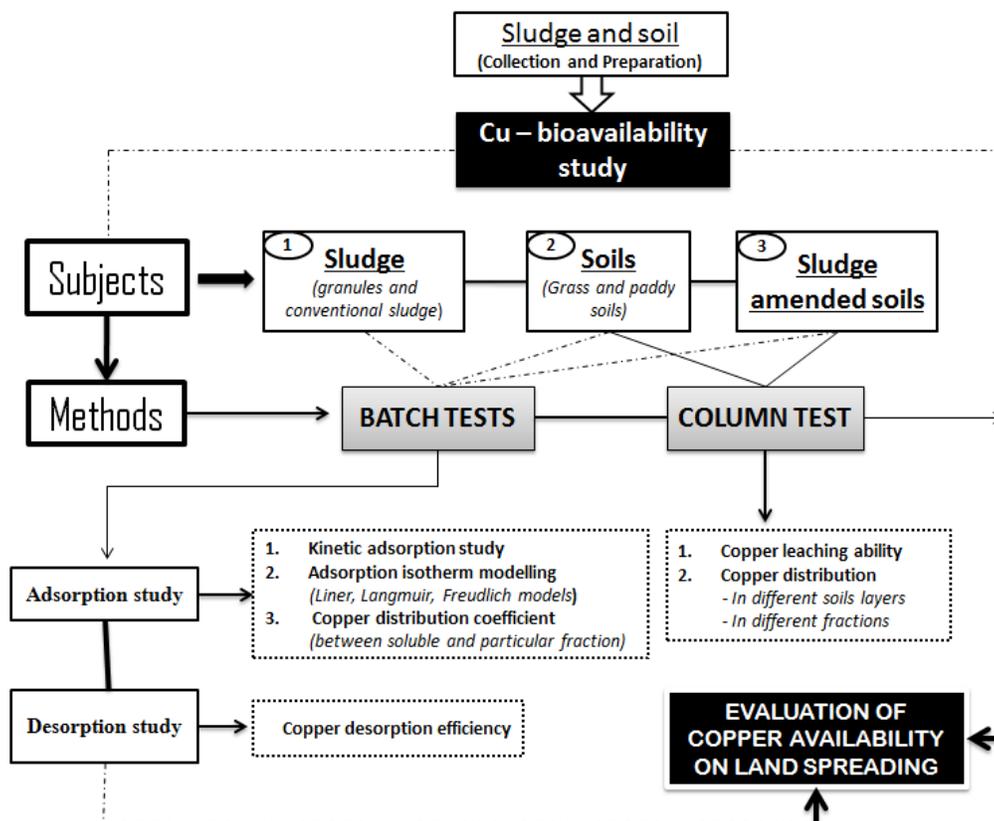


Figure 31: Summary diagram of the Cu adsorption/desorption experimental process on the sludge studies

III.1. Sludge and soil collection and preparation

III.1.1. Sludge preparation

Cu adsorption/desorption was conducted in several kinds of conventional sludge which contains: digested sludge (BD), limed sludge (BCH), composted sludge (CPT, CPC), thickened sludge (BE), reed bed sludge (LR), centrifuged sludge (BC), centri-dried sludge (BCD) and filter-pressed sludge (BFP) (Table 12). Granular sludge, which contains aerobic from SBR lab-scale reactor and anaerobic granular sludge from paper industrial wastewater treatment plant, were stabilized at laboratory by centrifugation. This process was conducted as thickening process according thickened sludge from sludge treatment plants. Granular and conventional sludge was collected and dried at dried at 105°C in 24 hours, prepared for all experiments.

III.1.2. Soil collection and preparation

➤ Soil collection

2 top soil samples were utilized in this study to represent different land use types, namely: grass land and paddy land. As presence in Figure 32, grass soil (GS) was chosen at a basement meadow non-anthropized (without rearing or culture) in the commune of Isle (87-Limosin- France). In this region, the average



annual rainfall and temperature are 1060 mm and 10°C, respectively (Lachassagne, 2014). Paddy soil (PS) with no previous history of sludge application obtained from the commune of Phu My- Binh Dinh, at Southern Coastal agriculture zone of Vietnam where is covered by mountains or hills and the popular type of soil is sandy soil. The average annual rainfall and temperature at Vietnamese soil collection region are 1560 mm and 26°C, respectively.



Figure 32: Mapping the soil sampling regions

➤ **Soil preparation**

Soils were collected at 0 – 15 cm depth surface after plant residue or debits was removed. At the experiment site, soil was air-dried under shade at ambient temperature and crushed by mortar and by hand and sieved through 2 mm stainless steel screen. These steps eliminates the effects linked to the soil structure, making reproducible tests and comparable from the column to another. Sieved soil was kept in plastic prior to experiments and analyses.

➤ **Soil characteristics**

Various characteristic of soil, both physical and chemical properties, which influence leaching, adsorption, and decomposition of copper in soil, were analyzed in the laboratory analysis of INRA in Arras. All the methods described detail in (Lachassagne, 2014). The soil characteristics were shown in Table 18.



Table 18: Selected properties of the grass and paddy soil horizon (0-15 cm)

Soil properties	Grass soil (GS)	Paddy soil (PS)
Type of soil	Brunisols	Hydragric Anthrosols
<i>Grain size distribution</i>		
Clay (%)	24.2	8.1
Slit (%)	29.2	17.7
Sand (%)	46.6	74.1
Texture	Loam	Sandy loam
<i>Physico-chemical properties</i>		
pH	5.6	5.2
Total nitrogen (mg g ⁻¹)	0.280	0.511
Organic carbon (mg g ⁻¹)	34.70	7.18
Organic matter (mg g ⁻¹)	60.1	12.4
C/N	12.4	14
Cation exchange capacity - CEC (cmol kg ⁻¹)	17	1.59
Exchange cations		
Ca ²⁺ (cmol kg ⁻¹)	12	0.487
Mg ²⁺ (cmol kg ⁻¹)	3.9	0.107
Na ¹⁺ (cmol kg ⁻¹)	0.187	0.0315
K ¹⁺ (cmol kg ⁻¹)	0.28	0.0489
AL ³⁺ (cmol kg ⁻¹)	0.18	0.243
Fe ²⁺ (cmol kg ⁻¹)	0.0084	< 0.005
Mn ²⁺ (cmol kg ⁻¹)	0.0532	0.042
S ²⁻ (cmol kg ⁻¹)	16.4	0.674
CaCO ₃ (mg g ⁻¹)	< 0.1	< 0.1

III.2. Batch test for study Cu availability on sludge, soils and sludge amended soils

III.2.1. Kinetic study for Cu adsorption on sludge and on soils

The Cu kinetic adsorption study is first and important step to determine the equilibrium time of Cu adsorption/desorption on sludge or on soils. In this study, the kinetic conditions were applied similarly for studying Cu adsorption on sludge and on soil.



➤ **Cu kinetic adsorption on sludge**

The kinetic adsorption was conducted at three different L/S (liquid/solid) ratios of 100, 50, and 10 (L/kg), therefore, according to each kind of sludge, 0.5 g; 1.0 g and 5.0 g of sludge dried matter was mixed with 50 ml of copper solution in plastic bottles, respectively. A known quantity of copper solution (CuCl_2 , purity > 98%) at 10 g/L (< 2% of the total volume) is added at the beginning of the test to obtain initial copper concentrations at 10 mg/L and 100 mg/L. The tests are made at constant pH 7. Adjustment of pH is done by the addition of 0.01 M HEPES buffer (SIGMA, H4034-100g, purity > 99.5%), this concentration was prepared by adding 1.1915 g HEPES in 50 ml of each adsorption solution in plastic bottles. The plastic bottles were then agitated on a thermostat shaker bath at 180 rpm and ambient temperature; they are shaken in 8 hours. Then, the solution was centrifuged at the 6000g, 20 min and 4°C, the supernatants were filtered by cellulose nitrate membrane 0.45 μm and were added few drops of concentrated acid HNO_3 69%. All these solution were stored at 4°C until analysis by AAS or F-AAS for determination of copper concentration. The concentration of copper adsorbed in particular sludge is obtained by difference between the initial and residual of copper concentration in solution at equilibrium. The sorbed copper amount q_e (mg/gTS) was determined according to Equation 11:

Equation 11 $q_e = (C_{\text{total}} - C_e) \cdot \frac{V}{m}$

Where: C_{total} and C_e : are the initial and residual of Cu concentration at equilibrium, respectively (mg/L).

V : is the volume of adsorption solution (mL).

m : is the amount of dried sludge (g).

➤ **Cu kinetic adsorption on soils**

The kinetic of copper adsorption on soils was conducted for both of grass and paddy soil. For each kind of soils, 1 g of soils was mixed in 50ml of adsorption solution which was contained in plastic bottles at 100 mgCu/L. The mixing condition corresponds with L/S ratio of 50 (L/kg). The pH of testing solution was not controlled. The plastic bottles were then agitation (180 rpm, room temperature) during 8h. Finally, the solution was centrifuged at the 6000g, 20 min and 4°C, the supernatants were filtered by cellulose nitrate membrane 0.45 μm and were added few drops of concentrated acid HNO_3 69%. All these solution were stored at 4°C until analysis by AAS or F-AAS for determination of copper concentration. The amount of Cu adsorption on soil particulate was calculated by using the Equation 11.



III.2.2. Batch tests study for Cu adsorption ability on sludge, soils and sludge amended soils

➤ Calculation of the amount sludge amended on soil for batch test experiments

Due to the big number of sludge, digested sludge was chosen for study of copper availability on sludge application soils. The adsorption/desorption experiments were conducted on three sludge application rates: 3 tons, 30 tons and 150 tons DS sludge ha⁻¹. The amount of sludge amended soils was calculated by Equation 12.

$$\text{Equation 12} \quad \% S = \frac{A.100\%}{10000 .H .D}$$

Where: %S: percentage of sludge amended soils (%) or the gram of sludge applied on 100 gram soils

A: Sludge application rates (tons DS/ha)

H: soil horizon (m) – in this study H = 0.1 m

D: Density of soils (g/cm³)

➤ Batch test establishment

The copper adsorption on sludge, soils and sludge amended soils experiments was established in the similar range of copper concentration in adsorption solution (from 0 mg/L to 1000 mg/L). According to L/S ratios, a known amount (in gram) of sludge, soil or sludge amended soil was added in 50 ml of adsorption solution in plastic bottles. The experimental conditions of Cu adsorption on sludge, soil and sludge amended soil were detailed in Table 19. The batch test bottles were agitated at 180 rpm in 4h at room temperature (25°C). At the end of adsorption test, the solution was separated by centrifugation, filter and was acidified by some drops of HNO₃ 69% , then stored at 4°C for analyzing residual copper concentration maintained in solution at equilibrium.

Table 19: Several different conditions of Cu adsorption on sludge, soils and sludge amended on soils experiments.

Batch test study	Adsorption conditions		
	L/S ratios (L/kg)	Type of adsorption solution	pH control
Sludge	50	Distilled water	Adjust pH 7 by adding HEPES buffer
Soils	10	Distilled water	None
Sludge amended soil	10	Distilled water	None



➤ **Adsorption isotherm modeling**

In order to evaluate the metal adsorption capacity in sludge, it is important to establish the most appropriate correlation for the equilibrium curve. Several isotherm models have been tested in the present study, namely, Liner, Langmuir and Freundlich (Limousin et al., 2007). These modeling permit us to determine the copper adsorption capacity and copper availability on sludge, soils and sludge amended soils.

The quality of the isotherm fit to the experimental data is typically assessed based on the magnitude of the correlation coefficient for the regression; i.e. the isotherm giving an R^2 value closet to unity is deemed to provide the best fit.

The Langmuir model has the following form:

Equation 13
$$q_e = q_{\max} \frac{K_L C_e}{1 + K_L C_e}$$

With q_e is the amount of Cu ion sorbed at equilibrium per g of sludge or soil or sludge amended soil (mg/g), q_m is the maximal Cu sorption capacity of sludge or soil or sludge amended soil (mg/g), C_e is the equilibrium Cu ion concentration in the solution (mg/L) and K_L is the constant of equilibrium (L/mg) depending on temperature and the applied conditions. This equation can be linearized under the following shape:

Equation 14
$$\frac{C_e}{q_e} = \frac{1}{q_{\max}} C_e + \frac{1}{K_L q_{\max}}$$

If the equation of Langmuir is valid to describe the experimental results, it must verify the linearized shape of the basis equation, in system of coordinates $C_e/q_e = f(C_e)$, that will permit to obtain the constants q_m and K_L from the intercepts and slopes.

The Freundlich model has the form:

Equation 15
$$q_e = K_f C_e^{1/n}$$

Where q_e and C_e are as described before, K_f and n are the positive constants depending on the nature of system solute-sludge or soil or sludge amended soil and temperature. This equation can be linearized under the following form:

Equation 16
$$\log q_e = \log K_f + \frac{1}{n} \log C_e$$

If this equation is verified with data of sorption equilibrium, we must obtain a straight line in the system of coordinates $\log q_e = f(\log C_e)$, the slope and the intercept to the origin give the value of constants n and K_f respectively.



The liner model:

The liner model was applied when the ratio between the concentration of the compound remaining in solution and adsorbed on the solid is the same at any concentration. Thus, this model is often used as an easy – to – use approximation (for a narrow range of concentration or very low concentrations), according to the form:

$$\text{Equation 17} \quad q_e = K_d \cdot C_e$$

Where q_e and C_e are as described before, K_d is distribution efficiency (L/kg) which was determined as the slope of liner isotherm curve following the relationship: $q_e = f(C_e)$

This model needs to be indicated the concentration range of adsorbate, otherwise it could lead to erroneous conclusions. For example, if the solid has a limited quantity of adsorption sites, the isotherm could be nonlinear because of a possible saturation plateau.

III.2.3. Batch tests study for Cu desorption ability from sludge, soils and sludge amended soils

Desorption batch test were carried out for study Cu desorption ability on sludge, soils and sludge amended soils. The amount of sludge amended on soil for desorption experiment study was also calculated by the Equation 12.

For establishment copper desorption batch test study, a known amount of sludge, soils or sludge amended soil was added on 50 ml of desorption solution in plastic bottle. The different amount of adsorbent depends on the L/S ratios. The Cu desorption ability on sludge, soils and sludge amended soil was conducted at several differences of experimental conditions which were presented in Table 20. Then, all the bottles for desorption batch tests are shaken at 180 rpm at ambient temperature in 4 hours. Once equilibrium was reached, the samples were centrifuged and filtered at 0.45 μm , the aqueous phases were acidified with a few drops of HNO_3 69% before analysis.

Table 20: Several different conditions of Cu desorption from sludge, soils and sludge amended on soils experiments.

Batch test study	Desorption conditions		
	L/S ratios (L/kg)	Type of desorption solution	pH control
Sludge	50	NaCl 0.02 M	None
Soils	10	NaCl 0.02 M	None
Sludge amended soil	10	NaCl 0.02 M	None

The concentration of copper presented in two fractions (or phases): the particulate and the soluble fraction, was analyzed by flame or furnace atomic absorption spectrometer.



All studies were carried out in duplicate and the copper desorption percentage were calculated according to the:

Equation 18 % Cu desorption = $\frac{C_{Cu(S)} \cdot V}{C_{Cu(P)} \cdot m}$

Where: $C_{Cu(S)}$: is Cu concentration in soluble fraction after desorption test (mg/L);

$C_{Cu(P)}$: is Cu concentration in particular phase before desorption test (mg/gTS)

V: Volume of desorption solution (L)

m: Amount of dried sludge used for desorption test (g TS)

III.3. Column test for study Cu leaching ability on sludge land spreading

III.3.1. Sludge amended soil column leaching experiment

Leaching experiment were performed using glass cylinders (4.5 cm inner diameter, a 40.0 cm height) with 2-cm depth quartz sand was applied at the bottom to retain soil. Air-dried soil (< 2mm) was carefully added to the column in approximate 2 cm increments to maintain soil thickness of 15 cm. The extract mass of the soil was calculated from the weight of the column before and after filling. Sludge obtained following the implementation of the treatment process described above is used to be applied to the soil columns, was planned according for sludge application rate 3 tons DS/ (ha.year) (ADEME, 1999). The amount of sludge added and mixed in topsoil (0-2 cm depth) layer was calculated by using Equation 19:

Equation 19 $m(\mu\text{g}) = \frac{A\left(\frac{\text{kg}}{\text{ha}}\right) \cdot 10^9\left(\frac{\mu\text{g}}{\text{kg}}\right) \cdot d^2(\text{cm}^2) \cdot \pi}{10^8\left(\frac{\text{cm}^2}{\text{ha}}\right) \cdot 4}$

Where: **m**: amount applied per column (μg)

A: rate of application (kg/ha)

d: diameter of soil column (cm)

π: 3.14

After filling process, a piece of quantitative filter paper was placed on top of soil column. The column slowly saturated with artificial rainfall (CaCl₂ 0.01 M, pH 6.8) from the bottom until field-holding capacity was reached prior to leaching tests. Then, the packed and saturated columns were immediately started experiment. For leaching process, 393 ml artificial rain was dropped on the top of column during 48 hours and repeated 5 times to simulate a one year rainfall in the Limousin area. Leachate following each leaching episode was collected in plastic bottles by gravity flow under the column. All tests were conducted in the darkness at ambient temperature (Figure 34 and Figure 33). Moreover, control soils were performed without sludge application.



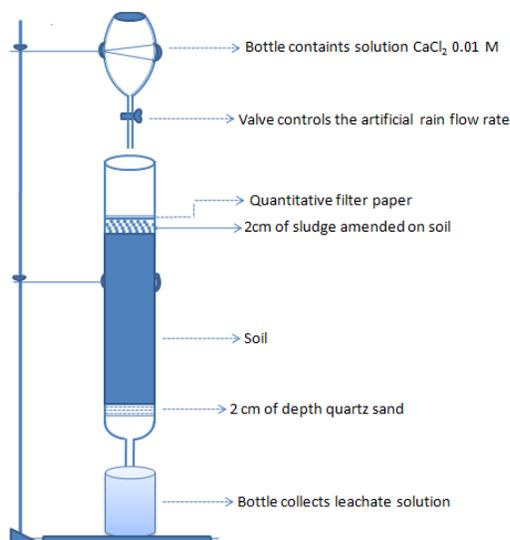


Figure 33: Leaching column experiment

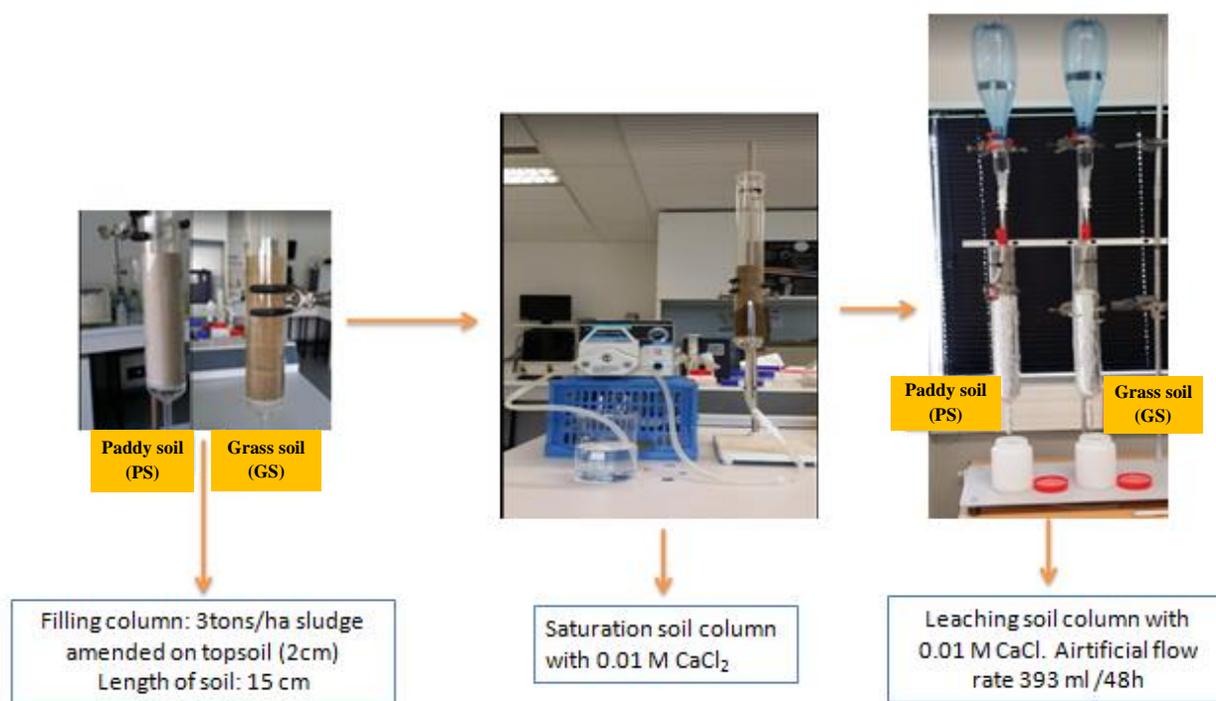


Figure 34: Photos of the establishment leaching test on sludge amended soil columns.

III.3.2. Soil leachate analysis

Each leaching treatments were carried out in duplicated (2 replicated x 2 soils x 11sludge). Five bottles of leachate (one bottle contains varied between 752.8 and 835.9 ml leachate) were selected for one leaching column, which were equivalent to average annual rainfall statistics in Limousin region. A part of each



leachate was acidified with concentrated HNO₃ (ultrapure) for determination of Cu amount in leachate then copper leaching percentages.

For one soil column, the Cu leached percentage in leachate after every two days of collecting was calculated by the Equation 20:

Equation 20
$$\%Cu_i = \frac{C_i \cdot V_i}{\sum_{i=1}^5 C_i V_i} \cdot 100\%$$

Where: %Cu_i: leaching desorption efficiency in each of five leachate (%)

C_i: is Cu concentration in each of five leachates (mg/L or µg/L)

V_i: is volume of each leachate (ml or L)

Other part of leaching solution was kept untreated for the determination of other parameters: pH, conductivity, redox potential, turbidity, TSS, dissolved organic carbon (DOC), UV adsorption at 254 nm, and fluorescence excitation-emission matrices (EEM) which were constructed from a series of emission spectra up to 550 nm by varying the excitation wavelength from 220 to 450 nm in 5 nm increments. All the analysis method was listed in Table 21:

Table 21: Sample analysis method

Parameter	Methods
pH	Multi 9430
Conductivity (EC)	Conductivity Meter LF 538
Redox potential (RP)	Multi 9430
Turbidity	Turb 550 IR
UV absorbance at 254 nm	UV-1700 Shimazu
dissolved organic carbon (DOC)	TOC – TekmarDohmann
Fluorescence EEM	Shimadzu RF-5301 PC
Copper	F-AAS

At the end of the test, for each column corresponding to each kind of sludge samples, volume of leachates collected during the test were mixed and characterized with the same leachate episode characteristics to determine the effect of each parameter for copper availability on sludge amended soil column. The copper leaching ability in total leaching solution is determined by Equation 21:



$$\text{Equation 21} \quad \% \text{ Cu leaching} = \frac{C_{\text{Cu (leachate)}} \cdot V}{C_{\text{Cu (soil)}} \cdot m_{\text{soil}} + C_{\text{Cu (sludge)}} \cdot m_{\text{sludge}}} \cdot 100\%$$

Where: $C_{\text{Cu (leachate)}}$: is copper concentration in composite leachate solution (mg/L)

V : is volume of composite leachate solution (L)

$C_{\text{Cu(Soil)}}$: Copper concentration in the soil (mg/g)

m_{soil} : Amount of soil in the column (g)

$C_{\text{Cu(sludge)}}$: Copper concentration in sludge (mg/gTS)

m_{sludge} : Amount of sludge amended on soil column (gTS)

➤ **Column dismantling and soil sampling extraction and analysis**

At the end of the experiment, the column was placed horizontally into sections of 0-5 cm, 5-10 cm, 10-15 cm, which were then air dried at ambient room temperature. Each soil column section was stored in plastic bags, the soil was then manually homogenized by shaking, sieved retaining the fraction < 1mm, and then weighed. The percentage of copper distribution in different soil layers was calculated based on Equation 22:

$$\text{Equation 22} \quad \% \text{ Cu}_{(D)} = \frac{m_{\text{Cu (L)}}}{m_{\text{Cu (T)}}} \cdot 100\%$$

Where: $\% \text{Cu}_{(D)}$: is percentages of copper distribution in each soil layers

$m_{\text{Cu (L)}}$: is amount of copper in each soil layer (mg)

$m_{\text{Cu (T)}}$: is amount of copper in soil column (mg)

Besides, copper distribution in different fraction in each soil layer was extracted by BCR-sequencing extraction method. According this method, the copper speciation is classified into four species: the acid-soluble fraction (F1), the reducible fraction (F2), the oxidizable fraction (F3), and the residual fraction (F4). The oxidizable fraction (F3) represents the metals bound to organic matters and sulphides and the residual fraction (F4) represents the portion of metals bound to silicate lattice or crystalline iron and manganese oxides with less mobility and potential bio-availability. In the present work, the samples were evaluated by means of the BCR- sequential extraction methods (II.5.3 – Part 2 – page 101).



Part 3: RESULTS & DISCUSSIONS





I. Chapter 1. Comparison between conventional activated sludge (CAS) and granular sludge originated samples: Insight into the sludge structure from macroscopic to microscopic considerations.

Numerous studies have shown the influence of the properties of the sludge matrix in the interactions with heavy metal during the adsorption/desorption processes (Ong et al., 2013; Bolan et al., 2014a; Laurent et al., 2010). There are different mechanisms convoluted in the interaction of heavy metals with sludge, which include physical processes like entrapment of metal ions within sludge matrix (Guibaud et al., 2005), adsorption of metal ions on the sludge surface (Ramrakhiani et al., 2016), diffusion of metals due to interface of heavy metals with negative charged consortium surface (Wei et al., 2016), Chemical process like precipitation of metals occurs. They are affected by pH values and sludge component such as carbonate (CO_3^{2-}), phosphate (PO_4^{3-}), sulfur (S^{2-}) or sulfate (SO_4^{2-}), etc, (Xu and Liu., 2008; Bartacek et al., 2008). Extracellular polymeric substances (EPS) components also play a vital role in heavy metal adsorption/desorption by effecting the complexation formation (Li and Yu, 2014).

Thus, the study of the fate of heavy metal in case of Cu in sludge during treatment process not only depends on the determination of the sorption/desorption constants but also on the effect of sludge matrix characteristics on Cu availability.

In this chapter, 9 sludge samples coming from conventional activated sludge systems and two granulate sludge samples were characterized (Figure 35). All the samples were collected in full scale installation, except aerobic granular sludge which was specifically produced during this study (Appendices 1). The interest of this work lies in the fact, originally, it was possible to compare in this study, not only stabilization treatment (digestion, liming, composting) but also sludge structure (CAS sludge versus granular sludge). These kinds of information are indeed really scarce in the literature.

The sludge matrices obtained during the various treatments have been characterized using several techniques. All the techniques were chosen to bring useful information giving sense to interaction between particulate/soluble parts of the sludge for one part and trace metal element for another part (Figure 35).

In this chapter sludge characterization was intentionally presented so that the sludge structures appear gradually from macroscopic to microscopic scale. And from general composition (TS, N, P content....), more accurate description (chemical functional groups nature and localization, 3D fluorescence of the soluble phases) were determined.

All the methods for sludge characterization were detailed in part 2 of Material and Methods and are reminded on (Figure 35).



This chapter is the starting point of this work, and numerous references to the content of this chapter would be done in chapter 2 and 3 as support for interpretation of copper sorption/desorption ability during land spreading.

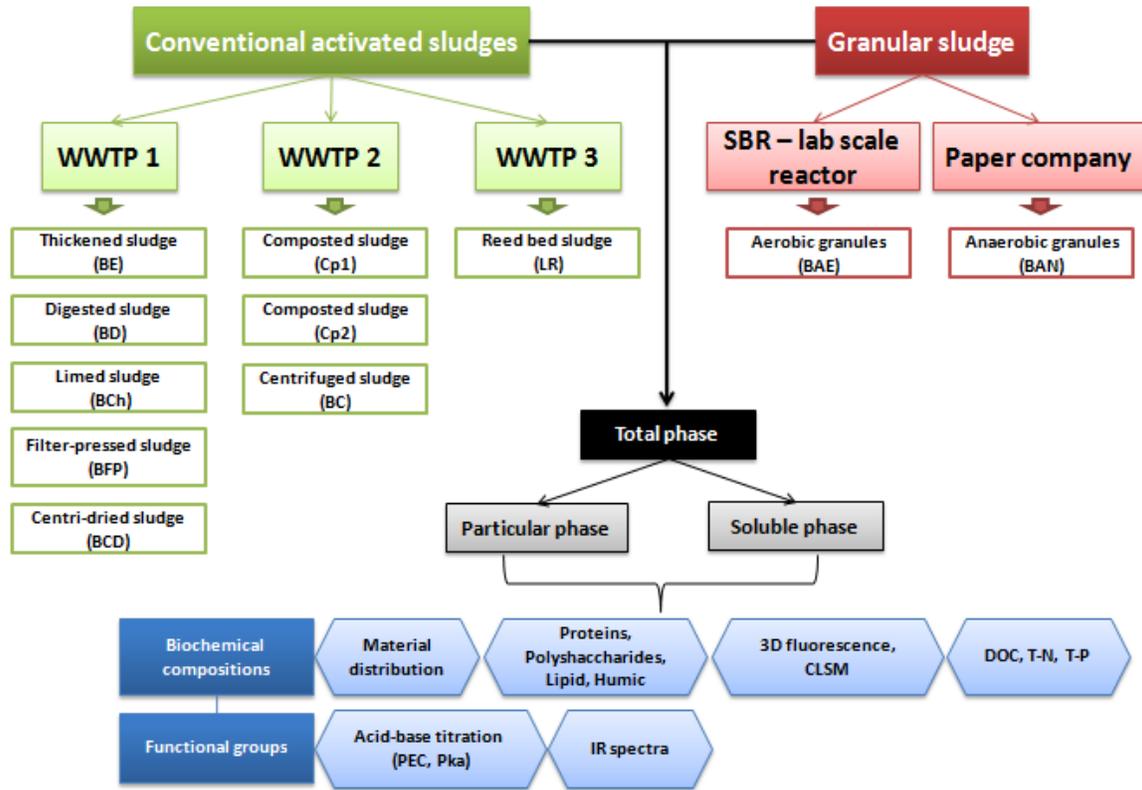


Figure 35: Summary of the sludge origin and analyzed methods carried out to various sludge characterizations.

I.1. Sludge characterization: general considerations.

I.1.1. Material distribution

Material distribution measurements are performed on each sludge sample at each step of the treatment. The results are presented in Table 22. The sludges studied are of different origins and undergo several treatments and/or stabilization, which modify their dry matter content (TS), but also the distribution between organic matter (VS) and mineral matter (MS).



Table 22: General sludge matrix characteristics after water and sludge treatment processes

Sludges		pH	Dried matter (TS) (g/L)	Organic matter (VS) (g/L)	Mineral matter (MS) (g/L)	% of VS	% of MS
Conventional activated sludge	BE (WWTP1)	6.88	1.16	0.87	0.29	74.92	25.08
	BCh (WWTP1)	12.89	1.67	0.97	0.70	58.31	41.69
	BD (WWTP1)	7.14	0.96	0.65	0.31	67.41	32.59
	BFP (WWTP1)	8.29	7.93	5.30	2.62	66.99	33.11
	BCD (WWTP1)	7.23	21.98	14.5	7.48	65.97	34.03
	BC (WWTP2)	5.83	8.18	6.40	1.78	78.23	21.77
	Cp1 (WWTP2)	7.08	15.34	8.66	6.68	56.44	43.56
	Cp2 (WWTP2)	7.20	14.31	8.07	6.24	55.58	44.42
	LR (WWTP3)	8.12	3.57	2.54	1.03	70.09	29.91
Granular sludge	BAN	6.80	91.19	78.58	12.61	71.65	28.35
	BAE	8.50	3.34	2.85	0.49	85.32	14.68



Material distribution was supposed to be one of the main important values on the excess sludge stabilization. After dewatering and composting treatment, the concentration of mineral and volatile matters significantly increased and showed the highest values for centri-dried sludge (BCD). By evaporation, 30% of water content in BFP sludge was removed, therefore, BFP also present high material distribution in sludge. Besides, the addition of green waste in composted sludge (Cp1 and Cp2) enhanced dramatically the mineral content in sludge with around 44%. Moreover, the addition of lime (with 30% $\text{Ca(OH)}_2/\text{gTS}$) in thickened sludge increased slightly the mineral content in BCh compared to BE, while the volatile content of BCh and BE is quite equal.

Sludge drying reed beds are a robust method for dewatering and stabilization of sludge. In our study, reed bed sludge (LR) also presented considerably material contents in sludge.

Furthermore, by comparing granular sludge and conventional sludge, BAE showed identically higher volatile value (85.32%) than BAN and other conventional sludge. However, the mineral contents in granule were lower than that in dewatering and composting sludge.

Volatile values normally indicate the organic matter content distributed in sludge. This kind of material often have high affinity for adsorption of heavy metal and especially Cu (Zhou et al., 2013a). In addition, the ion exchange mechanism during metal ion adsorption/desorption also effectively occurs on sludge with large content of mineral material. Therefore, sludge with high VS and MS values could be owned high adsorption capacity with metal ions. In the case of this study, dried, composted and granular sludge could be predicted for high adsorption capacity with copper ion in solution.

I.1.2. Dryness and pH of sludge

Dryness and pH are very important parameters as they greatly influence soil amending quality.

First of all, the **dryness** of sludge is important information because it greatly impacts the cost for sludge transportation and valorization. From this point of view the more the dryness is important the more the cost associated to transportation and valorization could be decreased. The dryness of the different sludge samples is presented on Figure 36. Concerning conventional activated sludge systems, composted (Cp1 and Cp2) and centri-dried sludge (BCD) exhibited the most important dry matter content. These results are not surprising when considering the literature on composting and the efficiency of centridry process.

From this point of view it is very interesting to observe that without any stabilization or drying treatment, the dryness of the granular sludge is very high compared to thickened (BE), limed (BCh), or digested sludge (BD). Thus the step of dewatering could be not necessary or drastically reduced for granular sludge, then inducing a reduction of the cost of sludge treatment.



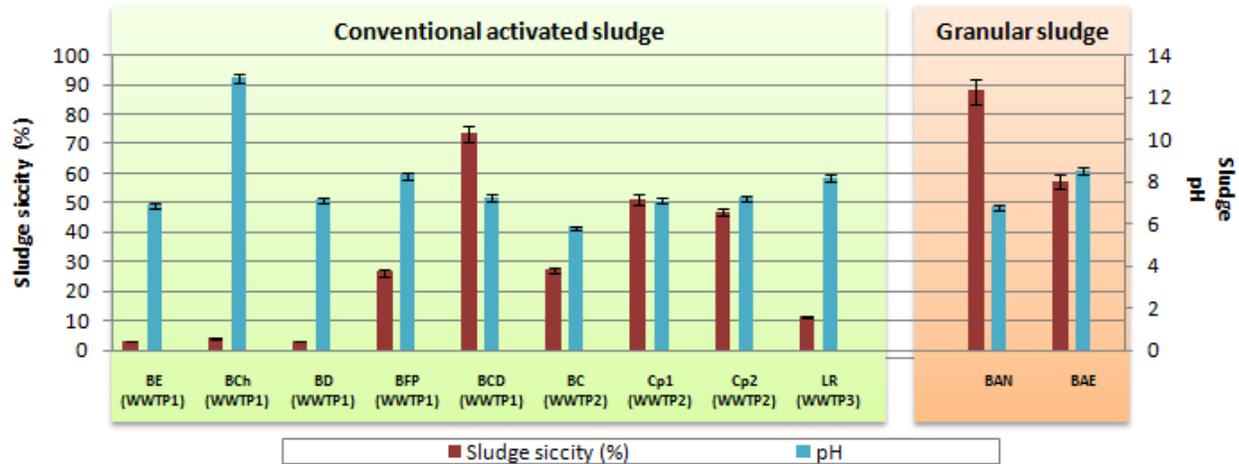


Figure 36: The pH and dryness values of different sludge

Other important characteristic of sludge is **pH** values.

The determination of pH plays a critical role in the wastewater and sludge treatment process. Extreme levels, presence of particulate matter, accumulation of toxic chemicals and increasing alkalinity levels are common problem which were often linked to pH values. pH is directly influenced by sludge stabilization treatment physical/chemical or biological. The pH variations of sludge samples varied from 6 to 8, except as waited, for limed sludge (BCh) which pH is equal to 12 (Figure 36).

The addition of limed in (BCh) and (BFP) samples led to increase alkalinity, especially for BCh sample (pH values equal to 12.9). Besides, reed bed sludge (LR) and aerobic granular sludge (BAE) also presented basic pH values which were probably due to the organic matter mineralization (for LR) and/or denitrification process in sludge reactor (for BAE).

According to French regulation (Decision of January 8th, 1998), sludge cannot be landspreaded on soil which pH is lower than 6 except if some conditions are simultaneously completed: sludge pH higher than 5 and liming.

1.1.3. Amending and fertilizing value (N and P)

Sewage sludge are also known to be rich in nutrients (nitrogen and phosphorus) that are beneficial for plant growth and gave better yield when sludge was applied for spreading in agricultural activities. For these purposes, sewage sludge is considered as suitable and the use of sewage sludge as a fertilizer decreases the requirement for commercial fertilizer.

The total nitrogen (T-N) and total phosphorus (T-P) content of sludge are reported on Figure 37.



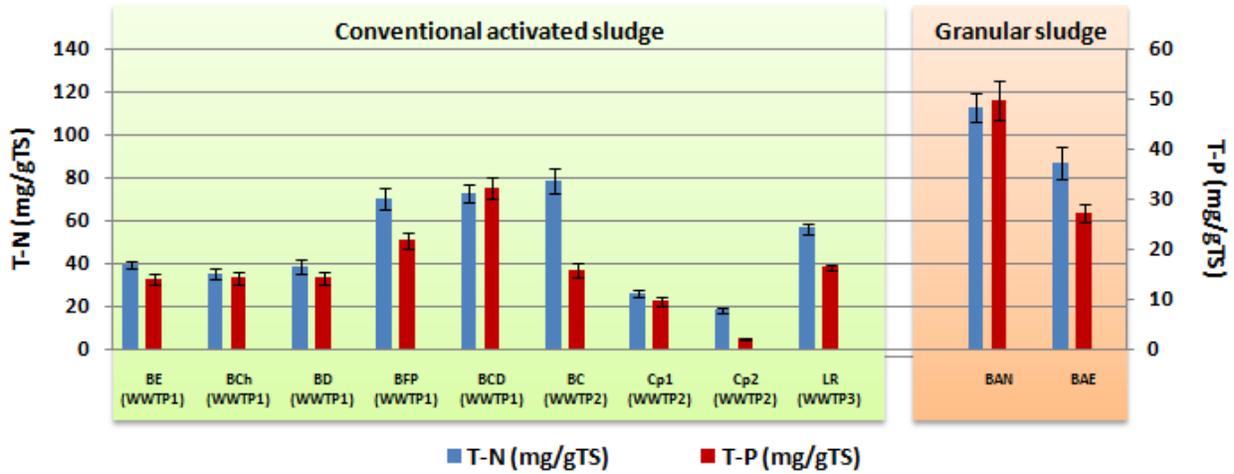


Figure 37: Total nitrogen (T-N) and total phosphorus (T-P) concentration in different sludge.

Considering on the one hand the nitrogen content in the samples coming from conventional activated sludge systems, it clearly appears that dewatering (centrifugation, filter press, bed drying, centri-dry) is the best way to keep interesting amount of nitrogen, on the other hand, granular sludge exhibited more important nitrogen concentrations compare to other sludge sample. For example, BCD sample has a high content of organic matter including high total nitrogen (72.54 mg/gTS) and phosphorus (32.25 mg/gTS).

Sludge stabilization by digestion (BD) or reed bed (LR) is not detrimental to N and P content compare to composting (Cp1 and Cp2).

Figure 37 also showed that composting stabilization process led to very poor phosphorus content compare to other sludge treatment processes. Considering the other sludge sample, originated from CAS systems, the range of P content is quite comparable and comprises between 2 and 20 mgP/gTS. One process led to important P content in CAS, it is the centridry sludge (BCD). This content is comparable to granular sludge phosphorus content and is quite high around 30 mgP/gTS.

In the case of granular sludge, the N and P content in anaerobic granules is reported but cannot be compared with other sludge sample, due to the fact that anaerobic granules were created from paper mill treatment and not from domestic wastewater treatment. In the case of aerobic granules, N and P also accumulated to a lower extend but the values of N and P content in aerobic granules were in the high range compared to centrifuged, filter press, reed bed and centridry sludge.

From these results it can be concluded that the granulation process is thus an interesting alternative to increase fertilizing value of sludge from wastewater treatment.

Therefore, the associated input of N and P in case of sludge landspreading at a level in accordance with French regulation is reported in Table 23. As waited, aerobic granular sludge led to the more important



inputs of N and P in the soil (results are lower than BAN, probably related to the origin of the effluent). Concerning CAS sludge sample, BCD sludge led to comparable results after a very costly process.

Table 23: The potential amount of N and P added to the soil for a sludge amending of 30 tDM/ha, corresponding to French regulation.

	Sludges	KgT-N/ha	KgT-P/ha
Conventional activated sludge	BE (WWTP1)	1183.5	416.7
	BCh (WWTP1)	1055.1	429.9
	BD (WWTP1)	1153.8	423.3
	BFP (WWTP1)	2109.6	649.2
	BCD (WWTP1)	2176.2	967.5
	BC (WWTP2)	2358.3	472.8
	Cp1 (WWTP2)	785.4	284.7
	Cp2 (WWTP2)	546.9	64.8
	LR (WWTP3)	1688.1	491.1
Granular sludge	BAN	3370.2	1513.7
	BAE	2600.4	814.5

I.2. Characterization of the particulate fraction.

I.2.1. Biochemical composition of sludge particulate fraction

The different sludge treatment processes have significant impact on biochemical sludge composition (Figure 38).



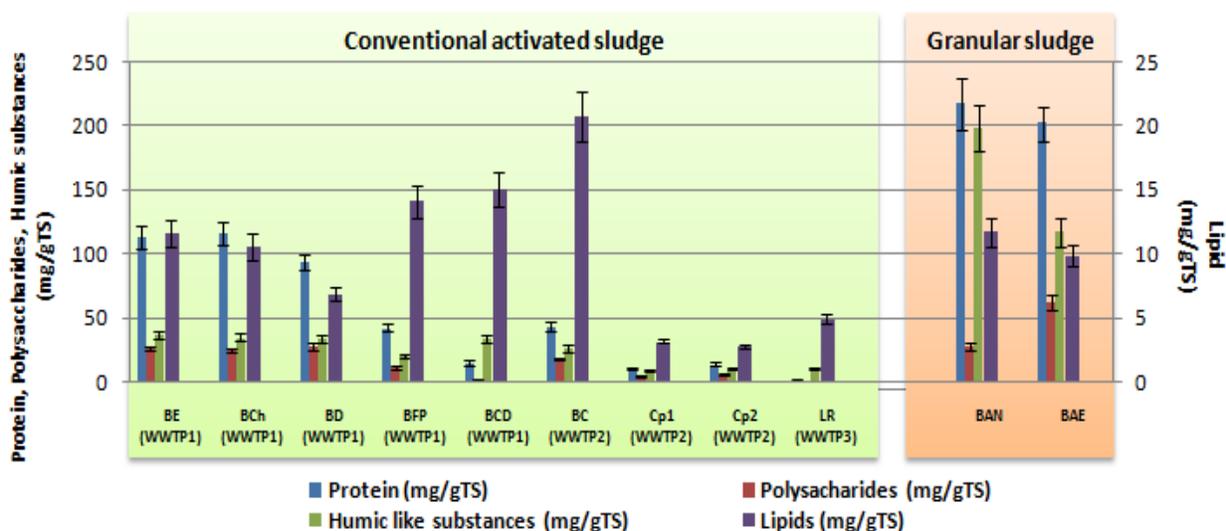


Figure 38: Biochemical component in particular phase of different sludge

In relation to the quantity of TS, protein sludge content is always higher than polysaccharides, “humic-like acid substances” and lipids, thus suggesting that protein is the major biochemical components of sludge.

Protein, polysaccharides, and humic-acid concentrations were higher in limed, thickened sludge and digested sludge. The release of extra-cellular polymer or biopolymer, primarily soluble proteins and polysaccharides, during digestion can explain those higher concentrations.

The dewatering process of CAS, centrifuged (BC), filter-press (BFP) and centri-dried sludge (BCD) led to higher lipid concentration compared to other CAS sludge samples and also to granular sludge samples.

Except in the case of lipid, BAE biochemical content is always higher in granular sludge compare to CAS. This result could be explained by the fact that during aerobic sludge formation, secretion of extracellular polymer components has an important role in the formation of granules. It makes the granular structure more stable, and protects them from erosion mechanisms of fluid hydrodynamics conditions. Inversely to the other biochemical compounds, the content of lipid in BAE is quite comparable to other sludge samples.

The titration and microscopic confocal measurement were implemented to give more precise information about chemical surface charge localization.

1.2.2. Confocal microscopy

Conventional biochemical characterization of sludge gave quantitative and qualitative information, but in the context of this work (study of interactions with copper), it is necessary to also determine their localization in the floc structure. The technique of confocal microscopy allowed localizing the biochemical components into the 3D structure of the floc. In the above pictures, specific fluorochromes



were used to visualize proteins (green fluorescent) and polysaccharides (red fluorescent) inside the sludge structure. For one kind of sludge, the observation was conducted on 10 samples with 5 z-stacking for each sample. The slice presenting in Table 24 was chosen at the out layer of sludge surface with the highest fluorescent intensities. All sludge samples were scanned at the similar conditions, and for each sludge sample, the standard deviation of fluorescence intensity stayed in the range 15% - 25%.

The results of confocal microscopy observation, for the different CAS sludge samples, are reported on Table 24. For some sludge samples, proteins and polysaccharides localization in the floc are quite comparable and for others is it completely different. At this stage, the technique was not developed enough for granular sludge because of their important size.

By adding $\text{Ca}(\text{OH})_2$ in BCh sludge, inorganic precipitation were created leading to a problem of absorption of the light in the sludge sample. However, the Ca^{2+} ion does not affect fluorescence observation by using the dye for organic components of protein and polysaccharides.

Confocal microscopy pictures were compared to the concentration of the corresponding biochemical components measured in the particulate fraction (Figure 358). The above pictures evidenced, that for some sample, even at lower concentration, a given biochemical compound can be localized at the sludge floc surface and could thus probably interact more with copper. The comparison between the biochemical and the microscopy allowed us to demonstrate that the biochemical concentration is probably not the only indicator explaining interaction between sludge and copper.



Table 24: CLSM spectra of protein and polysaccharides fluorescents in different conventional activated sludge

Sludges	Normal optical microscopy observation	Confocal Protein fluorescent	Confocal polysaccharide fluorescent	Sludge	Normal optical microscopy observation	Confocal Protein fluorescent	Confocal polysaccharide fluorescent
BE (WWTP1)				BC (WWTP2)			
BCh (WWTP1)				Cp1 (WWTP2)			
BD (WWTP1)				Cp2 (WWTP2)			
BFP (WWTP1)				LR (WWTP3)			
BCD (WWTP1)							



I.2.3. FTIR and titration.

Confocal microscopy allowed localizing proteins and polysaccharides in the sludge floc structure. FTIR and titration allowed having a more thorough insight in the chemical functions at the surface of the particulate phase of the floc. This information is fundamental as chemical function are driven copper interactions.

I.2.3.1 Infrared (IR) spectra

Fourier transform infrared (FT-IR) spectroscopy being a modern non-destructive analytical method, is more and more often used for the structure elucidation and quantification of large variety of organic, inorganic, and biological samples. One of the most advantages of IR spectroscopy is that all the chemical functions present in the sample are measured simultaneously, thereby simplifying and speeding up the analysis. The IR spectra and the main absorption bands detected in IR spectra of the particulate fractions of the different CAS and anaerobic granules are shown in Figure 39 and Table 25.

Table 25: Main absorption bands detected in IR spectra of the different sludge samples

Type of vibration	Functional groups	BE	BCh	BD	BFP	BCD	BC	Cp2	LR	BAN
-OH (3200-3400 cm ⁻¹)	Polymer component	+	+	+	+	+	+	+	+	+
-CH₂ (2930 cm ⁻¹)	Organic component	+	+	+	+	+	+	+	+	+
-CH₂ (2845 cm ⁻¹)	Organic component	+	+	+	+	+	+	+	+	+
-C=O (1720 cm ⁻¹)	Carbonic acid	+	-	-	-	-	-	-	-	-
-C=O and C-N (1640-1660 cm ⁻¹)	Proteins (amid I)	+	+	+	+	+	+	+	+	+
-C-N and N-H (1550-1560 cm ⁻¹)	Proteins (amid II)	+	-	+	+	+	+	+	+	+
-C=O (1400-1410 cm ⁻¹)	Carboxylate	+	+	+	+	+	+	+	+	+
-C-O-C- (1330-1660 cm ⁻¹)	polysaccharide	-	-	-	-	+	+	-	-	+
-C=O (1240 cm ⁻¹)	Carbonic acide	+	+	+	+	+	+	+	+	+
-OH (1040-1070 cm ⁻¹)	alcohol	+	+	+	+	+	+	+	+	+
-RR'C=CH₂ and RR'C=CH-R'' (800-900 cm ⁻¹)	Alkenes	-	-	-	+	-	-	-	-	-
P-O or S-O (650-700 cm ⁻¹)	Phosphate or sulphate	+	+	-	-	+	+	+	+	
		+ : Presence			- : Absence					



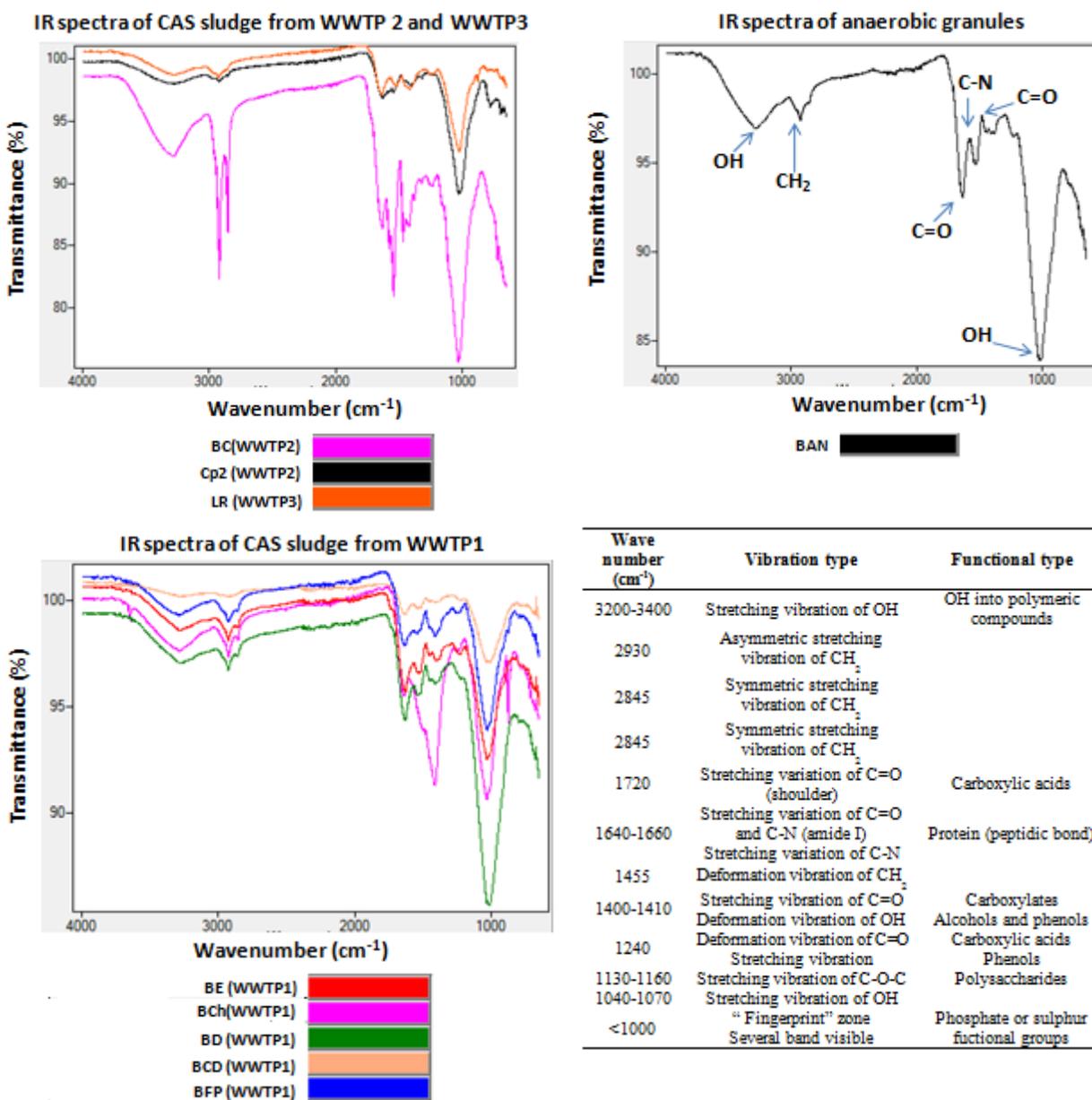


Figure 39: IR spectra of CAS sludge and anaerobic granular sludge and the main type of bonds and associated functional groups observed on IR spectra (Laurent, 2009).

The bands at 1640-1660 cm⁻¹ was assigned to the C=O stretching vibration of amide I. The N-H deformation vibration of amide II was observed at 1550-1560 cm⁻¹, whereas the peak at 1240 cm⁻¹ was due to the C-N stretching vibration of amide III. The bands at 1330-1660 cm⁻¹, and 1040-1070 cm⁻¹ were attributed to the C-O and C-C stretching as well as the C-O-C and C-O-H deformation vibration of the polysaccharide structure. The results indicated that the proteins groups could mix/bind carbohydrate in the structure of all sludge. More details of IR spectra for each kind of sludge were showed in the Table 25

The spectra showed a high intensity for three bands: 3200-3400, 1650 and 1034 cm^{-1} . The first corresponds to H-bonded OH groups and/or N-H groups, the second one arises mainly from benzoic compounds and the third one is produced mainly by ether linkages C-O-C. These characteristics confirm the presence of highly oxidized polymerized humic acid structures in composted sludge (Cp2), thickened sludge (BE), and digested sludge (BD).

The band 3600 cm^{-1} appeared only in limed sludge and was characteristic of the O-H free due to $\text{Ca}(\text{OH})_2$ addition.

The spectrum of the composting mixture is a superposition of all components and the most intense was broad absorption band at 1024 cm^{-1} . Occurrence of the characteristic bands of lignin: 1628, 1600, 1544 cm^{-1} evidenced the presence of wood chips (Grube et al., 2006). The intensity of these peaks appears quite similar on the reed-bed sludge that contributed to lignin which is residues of reed on the sludge (LR).

1.2.3.2 Acido-basic titration of the particulate phase

- Proton exchange capacity (PEC)

The PEC was plotted according to its distribution in both soluble and particulate phase (Figure 40) for clearer understanding. Strong differences in the distribution of PEC in soluble and particulate phases were observed in limed (BCh), thickened (BE), digested, (BD), reed bed (LR) and composted sludge (Cp1 and Cp2).

PEC was mainly distributed in the soluble phase of centrifuged activated sludge at about 60 %. This percentage increased to 70% for the filter-press sludge (BF), anaerobic (BAN) and centri-dried sludge (BCD). In composted sludge (Cp1 and Cp2), PEC was also mainly distributed in soluble phase, this can be explained by the oxidation process which increases the amount of PEC.

Opposite results were observed in digested sludge (BD), thickened sludge (BE) and limed sludge (BCh). The solid distribution of PEC is in majority localized in the particulate fraction. The proportion increased simultaneously with the increase of EPS content. For example, the protein content increased in the particulate phase of these samples (Figure 40) probably leading to an increase of the number of O-H links.

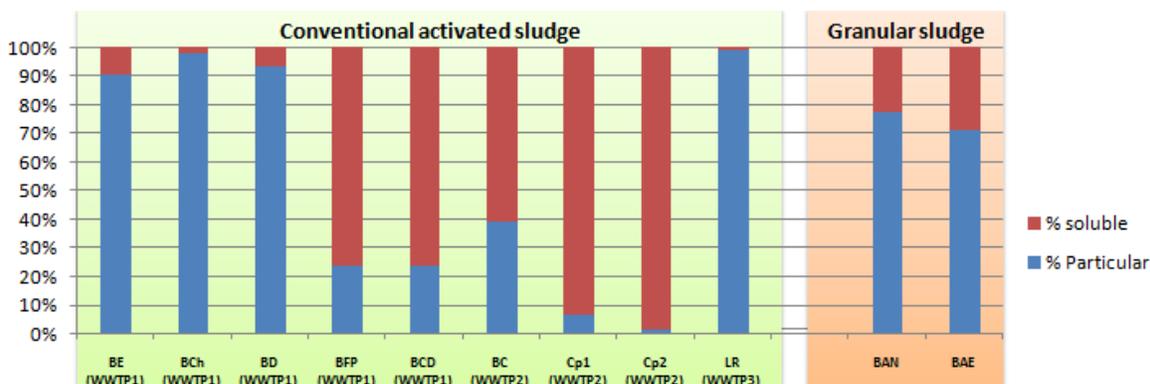


Figure 40: Distribution of PEC in particular and soluble phase of different sludge



- **Determination of pKa: nature of site present in the sludge**

Based on the acid-base titration method, four major types of functional groups have been identified and associated with their acidity constants: $pK_{a1} = 2.91$ to 4.3 and $pK_{a2} = 4.74$ to 4.85 are pK_a of carboxyl groups. $pK_{a3} = 6.27$ to 6.67 (phosphoric group) and $pK_{a4} = 8.25$ to 10.03 (amine or hydroxyl groups).

Figure 41 presented the three main kinds of sites:

- Carboxyl sites were presented as sum of two carboxyl groups with $pK_{a1} = 2.91$ to 4.3 and $pK_{a2} = 4.74$ to 4.85 . Depending on the nature of the carbon chain carrying the carboxyl groups, the pK_a values may vary.
- The phosphoric sites are present in proteins, humic substances and uronic acids.
- The amine/hydroxides sites are mainly coming from proteins, whereas hydroxyls are mainly derived from polysaccharides and humic substances.

The percentage of sites corresponding to amines/hydroxyls functions is higher in the composted (Cp2) and centrifuged sludge (BCD) compared to other CAS sludge samples and granular sludge. These sites will have a tendency to outcompete with negatively charged compounds or to complex (in case of amine groups) with cationic or positive groups or precipitate (in case of hydroxyl groups) with cations. The distribution of these functional groups is relatively comparable for limed (BCh), thickened (BE), filter-pressed (BFP) and reed bed sludge (LR).

In the particulate fraction of sludge sample (Cp2) and BD, 80% of the sites correspond to carboxyl groups. Moreover, composted (Cp2) also contained the highest percentage of phosphate groups (more than 40%), compared to other sludge, these groups being capable of reacting with metal cations (Laurent et al., 2010) or positively charged organic compounds (such as positive charge on surface of soils with sludge amendment).

In granular sludge samples, carboxyl groups also presented the highest percentages, followed by phosphoric and amine/hydroxyl groups. Larger carboxylic groups on the particulate phase of aerobic granular sludge (BAE) may indicate more potential ability to complex with Cu ion compared to anaerobic granules (BAN). Higher percentages of phosphoric groups in anaerobic granules could generate precipitate with cations.

The percentage of carboxyl and phosphoric groups in the particulate phase of granular sludge was lower than composted (Cp1) and digested sludge (BD) but higher than other sludge. The percentages of amine/hydroxyl groups of granular sludge were always lower than other sludge samples.



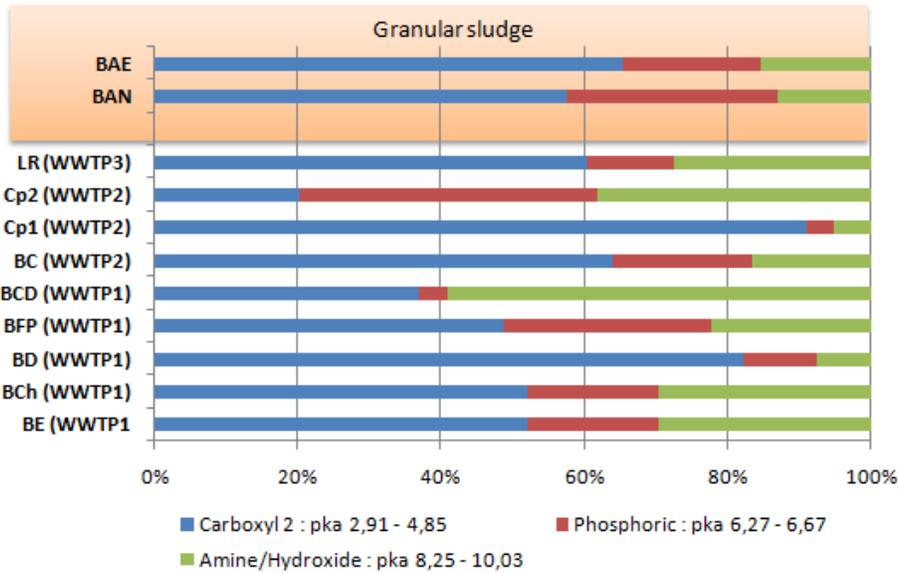


Figure 41: Percentage of carboxyl, phosphoric and amine hydroxide site in the particulate fractions of the different sludge samples.

I.3. Characterization of the soluble phase

I.3.1. Dissolved organic compounds (DOC)

Dissolved organic concentrations were measured after centrifugation and filtration of sludge sample. Characterization of sample soluble phase is very important at this phase could be “assimilated” to those generated from sludge in case of land spreading and precipitation simulation. The concentration of DOC in different sludges was presented in Figure 42. DOC concentrations are particularly high in BAN, Cp2, BCh, BCD, soluble phase, and to a lower extend in LR, BC, Cp1, BFP. The impact of DOC concentration on copper fate in soil is discussed later (next chapter).

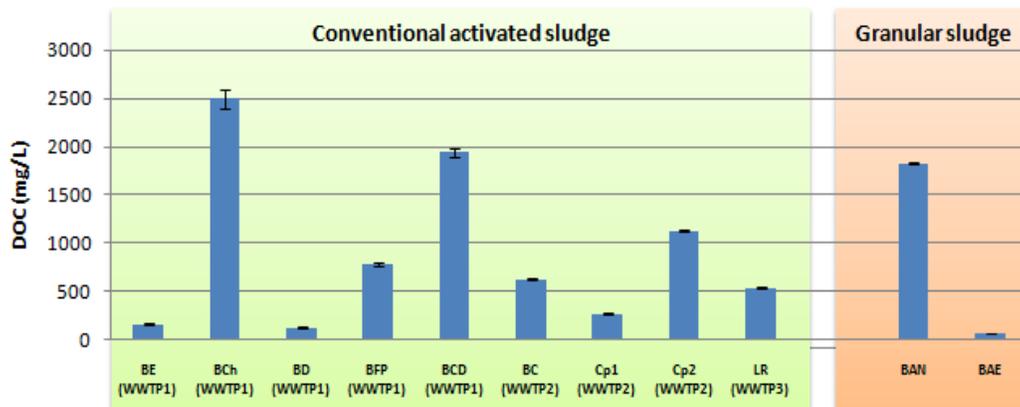


Figure 42: DOC concentration in different sludges



1.3.2. Biochemical composition

The composition of the soluble phase was investigated more accurately through biochemical composition measurement, titration and 3D fluorescence. The final objective is being to understand how this phase could interact with copper and to link those interactions with soluble phase specific characteristics. Therefore, while biochemical component in the particulate phase of sludge could allow interpreting the interaction between copper ions, and could be an indicator of the copper adsorption ability, the biochemical composition of the soluble phase could explain the solubility of copper ion due to complexation with soluble organic matter components.

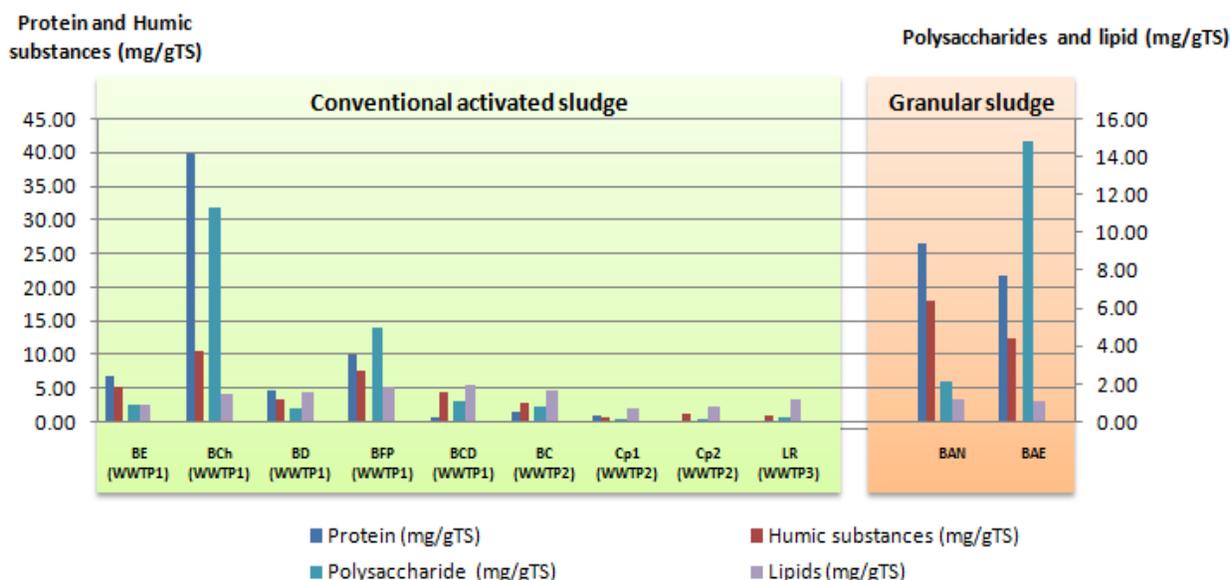


Figure 43: Biochemical components distribution in soluble phase of different sludge samples.

Among conventional activated sludge samples (Figure 43), the total soluble EPS concentration of BFP BCh, is always higher. BAE exhibited the most important soluble proteins and humic like substances concentrations compare to other sludge sample. Polysaccharides concentration was drastically higher in BAE sample.

As observed in the particulate phase characterization, the lipid content of the different sludge sample is quite comparable.

Surprisingly, the highest concentration of EPS in soluble phase was not correlated with the distribution of PEC in the soluble phase. In other words, high soluble phase EPS content does not mean high protonic exchange capacity.



1.3.3. Titration

The chemical functions present in the soluble phase were investigated and reported on Figure 44.

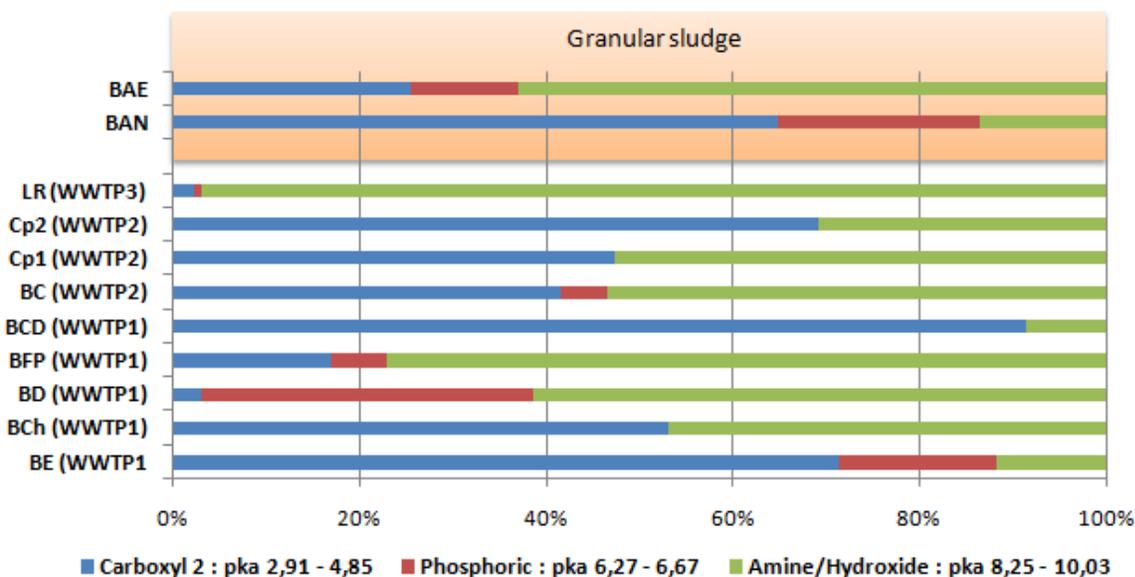


Figure 44: Distribution of different functional groups in soluble phase of different sludge

As observed for the particulate phase, functional groups distribution Figure 44 was made of three main types of sites: carboxyl groups associated with their pKa ($pK_a = 2.91$ to 4.85), phosphoric ($pK_a = 6.27$ to 6.67) and amine groups ($pK_a = 8.25$ to 10.03).

The highest percentages of carboxyl groups in soluble phase were associated to centrifuged (BC), thickened (BE), composted (Cp2) and anaerobic granular sludge (BAE) samples, representing more than 60% of the total distribution of functional groups.

Reed bed (LR), filter pressed (BFP), and aerobic granular sludge (BAE) contained high amine/hydroxyl functional groups in soluble phase with more than 65% of the total distribution of functional groups. The part of amine/hydroxyl functional groups is particularly high in reed bed sludge (LR) with approximate 90%.

The highest percentages of phosphoric groups distributed in soluble phase were observed for digested sludge (BD), followed by thickened (BE) and anaerobic granular sludge (BAN). This groups were not present in soluble phase of composted (Cp1 and Cp2), centrifried (BC), and reed beds sludge (LR).



I.3.4. 3D-Fluorescence.

Lachassagne et al., (2015) highlights that conventional EPS characterization (i.e., by colorimetric techniques) could not reveal any differences between two samples while 3D spectroscopic fluorescence can clearly explain different interactions with micropollutants.

3D fluorescence spectra are divided in 5 main zones. Each zone could be associated to specific organic matter (Part 2-II.2.6, page 92): Zone I (Trypsine proteins-like), Zone II (Tryptophane proteins-like), Zone III (microbial proteic-like by-products), Zone IV (Fulvic acid-like), and Zone V (Humic acid-like).

Fluorescence maximal intensity was identified for the different zone of the 3D fluorescence spectra (Appendices 2).

The distribution of relative fluorescence intensity (according to DOC concentration in soluble phase of sludge) in soluble fraction of different sludge was presented in Figure 45.

In some case, the distribution of polymeric components was determined by 3D-fluorescence method in accordance with biochemical characterization follows biochemical analysis (Figure 44) but sometimes it is not the case.

BAE and BAN exhibited high pick intensity in zone I and II corresponding to “protein like” fluorescence. This result is in accordance with proteins concentration in the soluble phase (Figure 44). Fluorescent density of proteins components in sludge is significantly higher than other components such as humic-like substances, excepted in composted sludge. This is consistent with analytical data in Figure 44, where the amount of humic-like acid and polysaccharide were lower than the amount of protein in both soluble and particulate phase. This suggests that protein structure decompose during composting and that the increase of humic substances concentrations was due to the process itself.

This distribution showed some discrepancies with biochemical composition distribution in soluble phase presented in Figure 45. In the case of BFP, for example, zone I and II had the highest relative intensity, while proteins concentrations measured by conventional biochemical methods were not the highest for this sample (Figure 44). It demonstrates that 3D fluorescence spectra can give very precious and more accurate information.



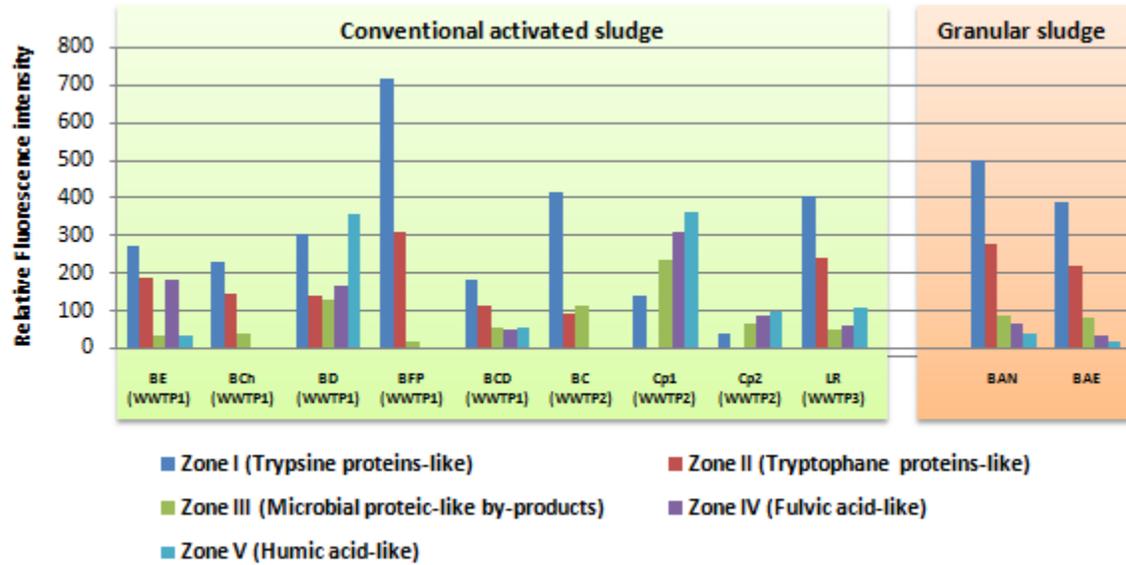


Figure 45: The distribution of relative intensity fluorescence in soluble fraction of different sludge

Centrifried (BCD), thickened (BE), digested (BD) and reed-bed sludge (LR), presented peaks in the five different zones. Fluorescence zone repartition is quite comparable for BAN, BAE and for most of other sludge samples, excepted sludge sample submitted to biological stabilization process like anaerobic digestion and composting. Digested sludge (BD) presents noticeable fluorescence intensity in each zone. In this sludge zone III, IV and V increased after digestion while zone I and II stayed constant. In case of composted sludge (Cp1 and Cp2), zone IV and V increased compared to other CAS sludge. This result is in accordance with the process of digestion and composting leading to humic like substances production.

I.4. Conclusion

The comparison between conventional activated sludge and granular sludge provided samples and the specific attention paid to insight into the sludge structure from macroscopic to microscopic considerations will be used in next chapter to argue the discussion concerning the interactions with copper.



II. Chapter 2: Characterization of interaction between copper and sludge/solid matrix before landspreading: Impact of stabilization process, sludge origin (CAS or granules) and kind of paddy/grass land soils

This chapter is specifically devoted to the study:

- of the biosorption/desorption properties of Cu in the sludge and soil matrix, separately and in mixing
- of Cu distribution in the sludge and soil after biosorption process.

Figure 46 recalls the methodology and the stage of study in this chapter.

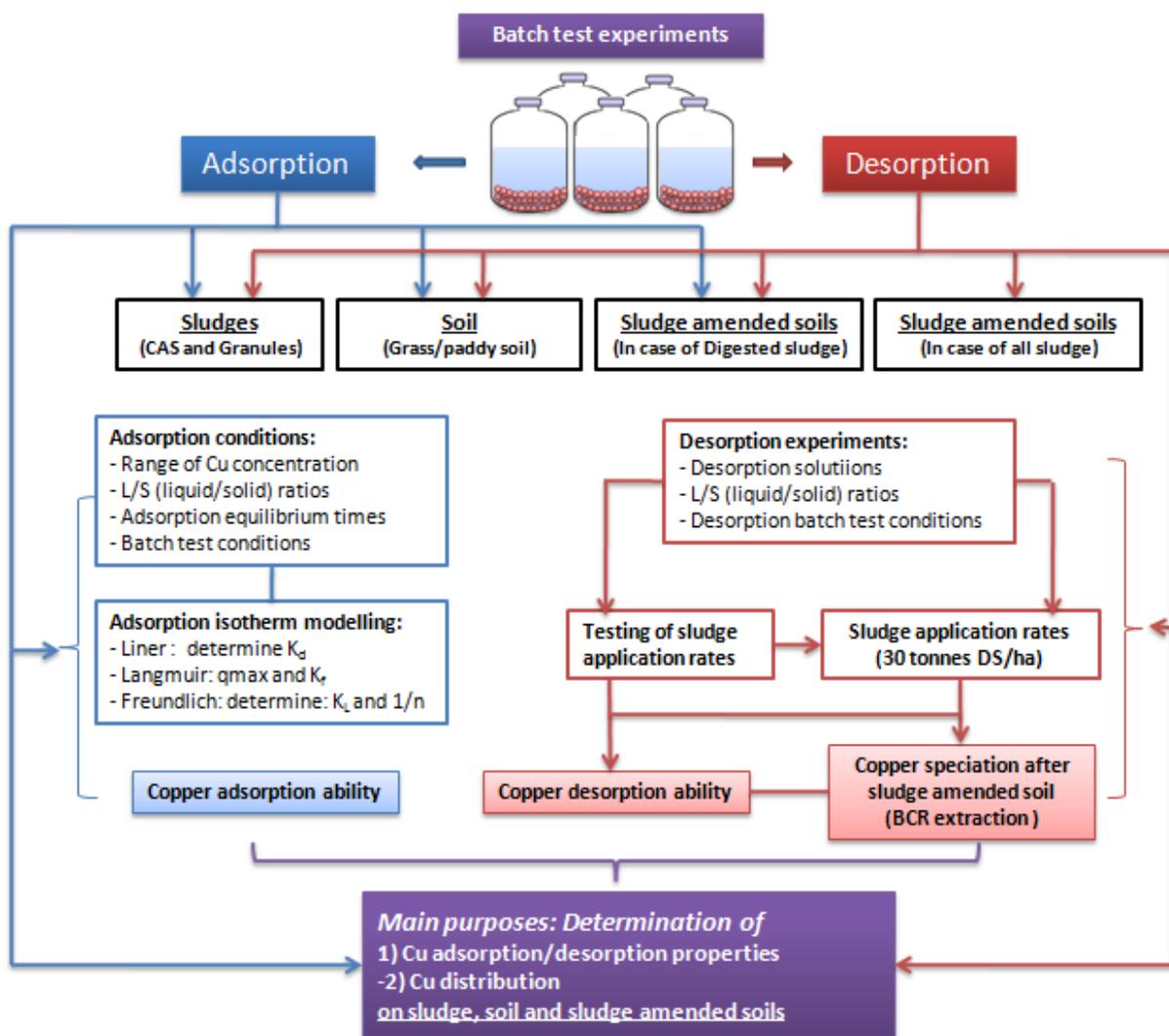


Figure 46 : Diagram of the experimental process for studying the Cu interaction in sludge and soil matrix



The first part of this chapter focused on Cu adsorption on conventional and granular sludge after stabilization process. 9 conventional sludge and 2 granular sludge (aerobic/anaerobic) samples were used in this study.

Cu biosorption/desorption processes in sludge and soils were investigated during batch test experiments. The kinetics and isotherms of the Cu biosorption were modeled for all the sludge samples. The factors impacting Cu kinetic adsorption on sludge were investigated such as: the range of Cu concentration, L/S (liquid/solid) ratio, sludge characteristics (physical-chemical properties), adsorption equilibrium time. Different models were fitting a part or the whole isotherms (i.e, linear, Langmuir, and Freundlich). Biosorption isotherms were established by using the equilibrium data and the isotherm constants in relation with the different models were determined.

In the same ways, the kinetic adsorption and sorption isotherms of Cu onto grass and paddy soils were also determined. Then, in order to explain the discrepancies of the obtained results, the sludge and soil characteristics were put in relation with Cu adsorption ability.

Secondly, Cu desorption was measured for all kind of sludge, soils and sludge amended soil following batch tests adsorption experiments (Figure 31).

In parallel, digested stabilized sludge were used to study different sludge application rates, L/S ratios on amended grass/paddy soils. At last, the Cu desorption experiments on sludge amended on soils were carried out at landspreading doses consistent with current agricultural conditions in France. The Cu distribution in sludge amended soil was analyzed by using BCR extraction method after desorption experiment.

The novelty of this approach was 1) the use of a combined approach between Cu adsorption, desorption and distributions and the number of sludge samples and the comparison of soils coming from completely different countries. 2) The final goal was to propose some hypothesis on the possible Cu desorption ability following the sludge application and to contribute to the debate regarding the nature and availability of Cu bearing components during agricultural sludge recycling or land spreading

II.1. Copper concentration and distribution in native sludge sample

Cu concentrations present in the nine conventional activated sludges and two granular sludges taken from different WWTPs and lab-scale reactor were analyzed by the F-AAS (see part 2 - II.5.2; page 100).The total Cu concentration and the soluble/particulate partition constant were determined. Moreover the speciation of copper was also elucidated for each sludge samples.



- **Total copper concentration in sludge**

The Cu concentration showed variations that reflect 1) the characteristics of the incoming wastewaters (urban domestic, rural domestic, industrial); 2) the kind of wastewater treatment (CAS or granular) and the kind of sludge stabilization treatment. The Cu concentrations in sludge were presented in Figure 47.

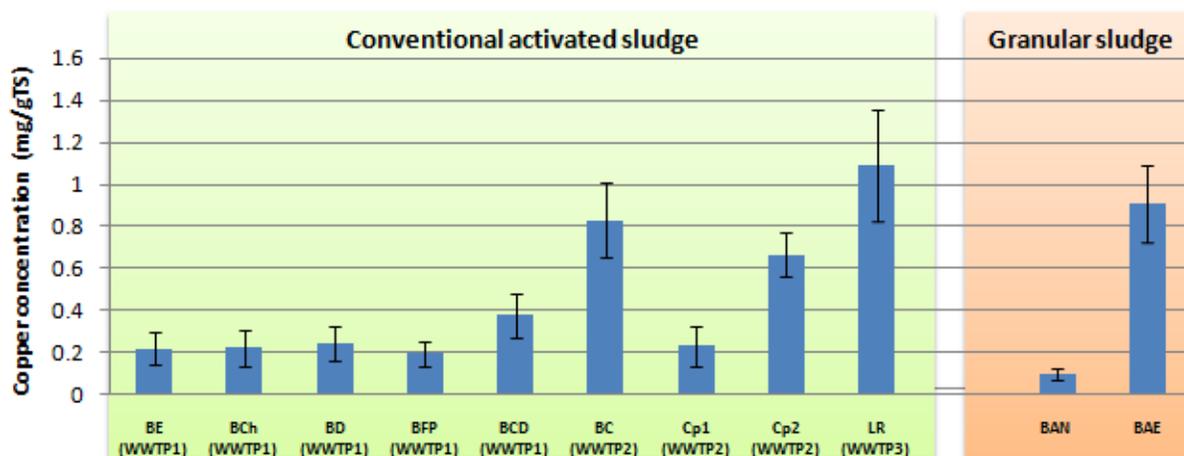


Figure 47 : Total Cu concentration in conventional and granular sludge

Conventional sludge:

Sludge samples from the WWTP1 generally showed lower Cu concentrations compared to the other conventional sludge samples taken from the other WWTP. BCD presented the highest Cu concentration (0.378 ± 0.103 g/kgTS), BD, BFP, BCH and BE had Cu concentration lower than 0.25 g/kgTS. These results are consistent with the conclusions of the ESCoMafor (2014) on the evolution of trace metal element contents in sludge in France, which indicated that the Cu concentration was usually lower than 0.350 g/kgTS.

In our study, the concentration of copper in BC and Cp2 which collected from WWTP2 is higher than in Cp1. The highest Cu concentration obtained in LR sludge from WWTP3 with 1.091 ± 0.264 g/kgTS. The large Cu concentration in reed bed sludge may due to the accumulation of Cu following the long retention time of sludge associated with sludge treatment in reed bed. (Kengne and Tilley, 2014; Stefanakis and Tsihrintzis, 2012) investigated that during reed bed sewage sludge treatment the concentration of metals in sludge typically increase as the organic matter is reduced through decomposition. They indicated that sedimentation, adsorption and precipitation (as metal oxides, carbonates and sulphites) are the primary mechanisms through which the gravel and sand layers trap and retain metals as they pass through the bed.



Granular sludge:

The Cu concentration in BAN from paper industrial WWTP was lower than that in BAE from SBR lab-scale reactor after 47 days of granulation. Compared to conventional sludge, while BAN had Cu concentrations (0.104 ± 0.018 g/kgTS) lower than all conventional sludge, BAE showed Cu concentration (0.911 ± 0.182 g/kgTS) higher than sludge almost CAS sludge but lower than LR sludge.

During aerobic granulation, the Cu concentration in synthetics wastewater stayed as trace amount with 0.05 mg/L. Under the anoxic condition, the pH values in reactor were closed to 8.5. It could be hypothesised that these conditions promoted Cu precipitation or accumulation in the core of granular sludge and increase significantly Cu concentration in granular sludge according to the sludge formation.

The Cu concentrations in conventional and granular sludge were also compared with French regulatory limits for trace metal elements in sludge land spreading. In present study, sludge copper concentrations were mostly within the regulatory limits of the standards considered for Cu concentration (1 g/kgTS), excepted for LR sludge. The results of the experiments showed low risk of heavy metal toxicity of sludge samples (in terms of the regulatory limits specified) and therefore a reasonably good potential for the sludge to be used for agricultural purposes. Out of the context of French regulation, the reed bed sludge should be noticed for the hazards when application for land spreading.

- **Copper distribution in the different sludge samples (sequential extraction)**

The distribution of Cu in the different fractions in the sludge allows predicting their mobility and bioavailability. In this study, the Cu distribution in four fractions: exchangeable fractions (F1), reducible fraction (F2), oxidizable fraction (F3) and residual fraction (F4) were determined by BCR extraction method (see part 2-II.5.3, page 101) and were presented in Figure 48.

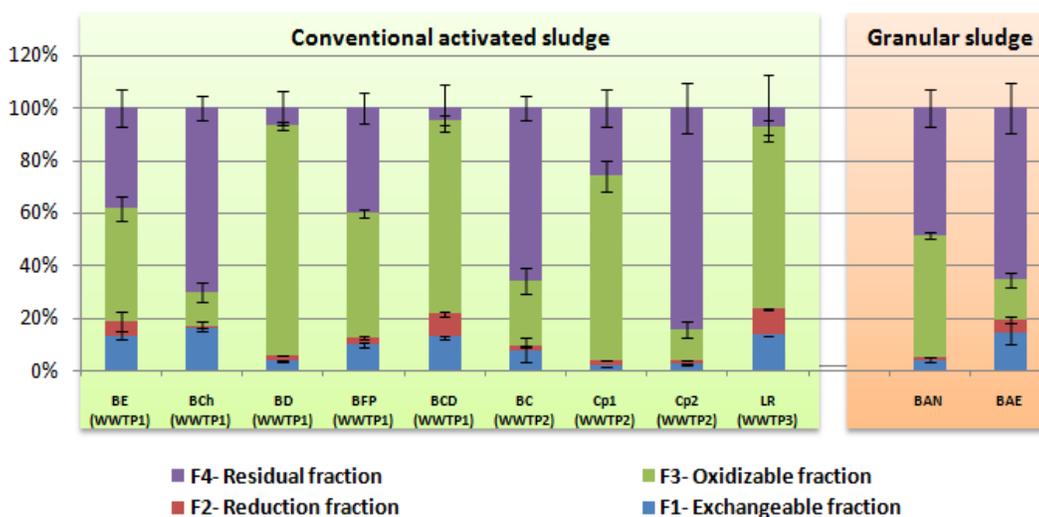


Figure 48 : Copper distribution in different fractions of sludge



Cu bound residual fraction (F4) was higher than 40% not only for some conventional sludge such as Cp2, BC and BCh but also for BAE - granular sludge. From the observation of Cu distribution in Cp1 and Cp2, it can be understood that, during composting, the addition of centrifuged sludge and green waste in Cp1 sludge, can enhance more Cu adsorption on organic matter components, which lead to higher Cu – bound organic fraction (F3) in Cp1 than that in Cp2. In residual fraction Cu was normally stable in forms of crystal structure.

Besides, Cp1, BD, BCD and LR sludge samples contained high amount of Cu in organic matter fraction (F3) with 70.27%, 73.92% and 69.10%, respectively. BCh and Cp2 presented very low Cu form in organic matter fraction (F3). Compared to BE, the adding lime with the ratio of 30% of $\text{Ca}(\text{OH})_2$ per gram of dried sludge facilitated the increase of the sludge pH and provided more amount of ion hydroxide (OH^-) which promoted the stable form of metal ion such as the precipitation. That led to increase the residual fraction (F4) of copper and to reduce the organic matter form in BCh.

Almost conventional sludge presented low percentages of Cu partition on reducible fraction (F2). The highest percentages of Cu – bound reducible fraction was measured in LR sludge with 10.09%, followed by BCD sludge with 8.75% and other conventional sludge with lower than 5.54% (BE). Low levels of the Cu-bound reducible fraction (F2) can be attributed to the low affinity of this metal to metals associated with manganese and/or iron (hydr)oxides.

Similarly, the Cu bound-exchangeable (F1) also showed low Cu distribution percentages. The copper distribution in exchangeable fraction ranged in the order of BCh > LR > BE > BCD > BFP > BC > BD > Cp2 > Cp1. In theory, heavy metal ions in exchangeable fraction were potentially bioavailable, especially under acidic condition. These fractions presented the risk of heavy metal release due to its easy mobility and transportation.

In case of granular sludge, while in BAE sludge Cu is almost distributed in residual fraction (F4) with high Cu distribution percentages (65.65%) and low Cu distribution in other fractions. in BAN sludge Cu was distributed in organic matter (F3) and residual fractions (F4). In BAN, only 1.16% and 3.97% of Cu components was found in reducible fraction (F2) and exchangeable fraction (F1), respectively.

II.2. General characteristics of soils

- **Physical and chemical properties of grass and paddy soils.**

Physical and chemical properties of grass soil (GS) and paddy soil (PS) were analyzed and are presented in the Table 26.

Soil pH was relatively low with a value of 5.6 and 5.2 for grass and paddy soils, respectively. Any soil with pH below 6.5 could increase metal solubility, resulting in phyto-toxicity to plants (Parkpian et al., 2002b). Grass soil contained 24.2% of clay; 29.2% of slit and 46.6% of sand, which is classified as a loam soil. Paddy soil contained 74% of sand, which is classified as sandy loam soil. Nitrogen application is necessary for supplying sufficient nutrients for plant growth in this soil. Base on the site-specific nutrient



management (SSNM) approach for fertilization of rice (IRRD, 2006.). A rice crop requires about 50 kg/ha of N fertilizer. It corresponds with approximate 3 mg-N/g PS in case of this study (Equation 12). Total nitrogen content of the paddy soil is 0.511 mg/g, which is considered low for rice growth (Parkpian et al., 2002b). In addition, the contents of organic carbon and organic matter of paddy soil are lower than that of grass soil which was collected on grass land region. The organic matter and nutrient contents in paddy soil is low because of high sand and low clay contents in this soil. The exchangeable cation in paddy soil is lower than that in grass soil. Soil with higher clay content such as grass soil has a higher cation exchange capacity (17 cmol kg⁻¹) (Parkpian et al., 2002b).

Table 26 : Summary characteristics of GS and PS (See part 2 - III.1.2, page 104)

Soil properties	Grass soil (GS)	Paddy soil (PS)
Type of soil	Brunisols	Hydragric Anthrosols
<i>Grain size distribution</i>		
Clay (percentage)	24.2	8.1
Slit (percentage)	29.2	17.7
Sand (percentage)	46.6	74.1
Texture	Loam	Sandy loam
<i>Physico-chemical properties</i>		
pH	5.6	5.2
Total nitrogen (mg g ⁻¹)	0.280	0.511
Organic carbon (mg g ⁻¹)	34.70	7.18
Organic matter (mg g ⁻¹)	60.1	12.4
C/N	12.4	14
Cation exchange capacity CEC (cmol kg ⁻¹)	17	1.59
Exchange cations		
Ca ²⁺ (cmol kg ⁻¹)	12	0.487
Mg ²⁺ (cmol kg ⁻¹)	3.9	0.107
Na ¹⁺ (cmol kg ⁻¹)	0.187	0.0315
K ¹⁺ (cmol kg ⁻¹)	0.28	0.0489
AL ³⁺ (cmol kg ⁻¹)	0.18	0.243
Fe ²⁺ (cmol kg ⁻¹)	0.0084	< 0.005
Mn ²⁺ (cmol kg ⁻¹)	0.0532	0.042
S ²⁻ (cmol kg ⁻¹)	16.4	0.674
CaCO ₃ (mg g ⁻¹)	< 0.1	< 0.1



Some reasons may explain the differences of physical and chemical properties between grass and paddy soil. Firstly, the temperature and rainfall have influence on the soil structures and components (Karmakar et al., 2016). The sandy loam-paddy soil contains high percentages of sand and very low percentage of clay. Under heavy annual rainfall condition (1650 mm), paddy soil is capable of quickly draining excess water but cannot hold significant amount of water or nutrients. The dissolved organic matter can be easily washed out through draining process. Besides, the rate of organic matter decomposition, evaporation and other chemical which easily transfer via the movement of gas and water are obviously increased at high annual temperatures (Karmakar et al., 2016). It could explain the low nutrient contents in paddy soil.

Moreover, mineralization of organic matter in paddy land will be more rapid under continual cultivation than under grass land or woodland (Zhou et al., 2013a). As in many other countries of South-East Asia, in Vietnam, field-grown crops are often rotated with foolded rice. This rotation has disadvantages. Wet puddling, necessary for foolded rice, affects soil structure, resulting in restricted water movement in the soil, making soil nutrient less available for rice growth (Huong et al., 2013). In such cases, the low soil fertility and low nutrient holding capacity of the paddy soil may be compensated by the repeated application of fertilizer or amending by materials with high nutrient contents such as sewage sludge in paddy land regions (Dung el al., 2003; Huong et al., 2013).

- **Cu content and distribution in soils**

The distribution of Cu in grass soil and paddy soil was determined by BCR extraction. According to the Table 27, the native Cu concentration was high in the grass soil compare to the paddy soil. The low Cu concentration in paddy soil may explain larger desorption efficiency as demonstrated in the next part of this chapter during desorption batch test experiments (II.6.2, page 164).

The Cu distribution in the two soils showed that the copper was mainly in the residual fraction (highest percentages) followed by the oxidant fraction, reducing and exchangeable fractions. No Cu – exchangeable fraction was found in paddy soil and this fraction presented the lowest percentages in grass soil (1.88%). The percentages of Cu in reducible and oxidizable fractions were higher in paddy soil (5.65% and 20.88%) than in grass soil (3.20% and 15.30%). As presented in Table 27, paddy soil is more acid than grass soil. The acidification of paddy soil can result in higher concentration of free Cu in soil solution and easily form organic complexes with organic matter content in soil. Hence, the amount of Cu – organic matter fraction in paddy soil can leach rapidly by dissolved organic matter during leaching process (Torri and Lavado, 2009).

The Cu distribution between soluble/particulate fractions was presented in Table 27. Both grass soil and paddy soil showed high Cu distribution percentages in particulate phase. Besides, percentages of Cu in particulate phase and Cu distribution coefficient of paddy soil are lower than that of grass soil.

Combination between Cu distribution in soluble/particulate phases and Cu distribution in different fractions indicated that Cu was almost in form of organic and crystallized form in particulate phase of soils.



Table 27 : Concentration and distribution of Cu in grass soil and paddy soil

Soils	Grass soil	Paddy soil
Total Cu concentration (mg/kg)	45.9	1.79
Cu distribution in soluble/particulate phases		
Percentage soluble	0.68	3.9
Percentage particulate	99.32	96.1
K_d (L/Kg)	1132	82.6
Cu distribution in different fraction (BCR extraction method)		
F1- exchangeable fraction (percentage)	1.88	ND
F2- reducible fraction (percentage)	3.20	5.65
F3- Oxidizable fraction (percentage)	15.30	20.88
F4- Reducible fraction (percentage)	79.63	73.48

(ND: non detected)

II.3. Modeling Cu sorption in different sludge matrix

II.3.1. Determination of biosorption equilibrium time

For determination of Cu biosorption ability on sludge, it was important to carry out the sorption kinetic tests. The kinetic study makes it possible to indicate the equilibrium time necessary for Cu sorption on the particulate phase of the sludge.

In this study, the time required for reaching equilibrium was not investigated for all kind of conventional and granular sludge. Samples from the three main waste activated sludge stabilization processes in WWTP were selected: liming, anaerobic digestion, composting for study equilibrium time according to the condition described in (part 2 - III.2.2, page 108).

pH of solution was controlled at pH 7 by HEPES buffer (Lachassagne, 2014; Laurent, 2009). Two initial copper concentrations (10 mg/L and 100 mg/L) were performed in order to observe the effect of initial Cu concentration on Cu sorption kinetic equilibrium. The effect of liquid to solid ratio (L/S ratio) on Cu kinetic biosorption was studied at three L/S ratios: 100; 50 and 10 (L/Kg). The relationship between the Cu sorption ability on sludge at experimental conditions was presented in Figure 49.



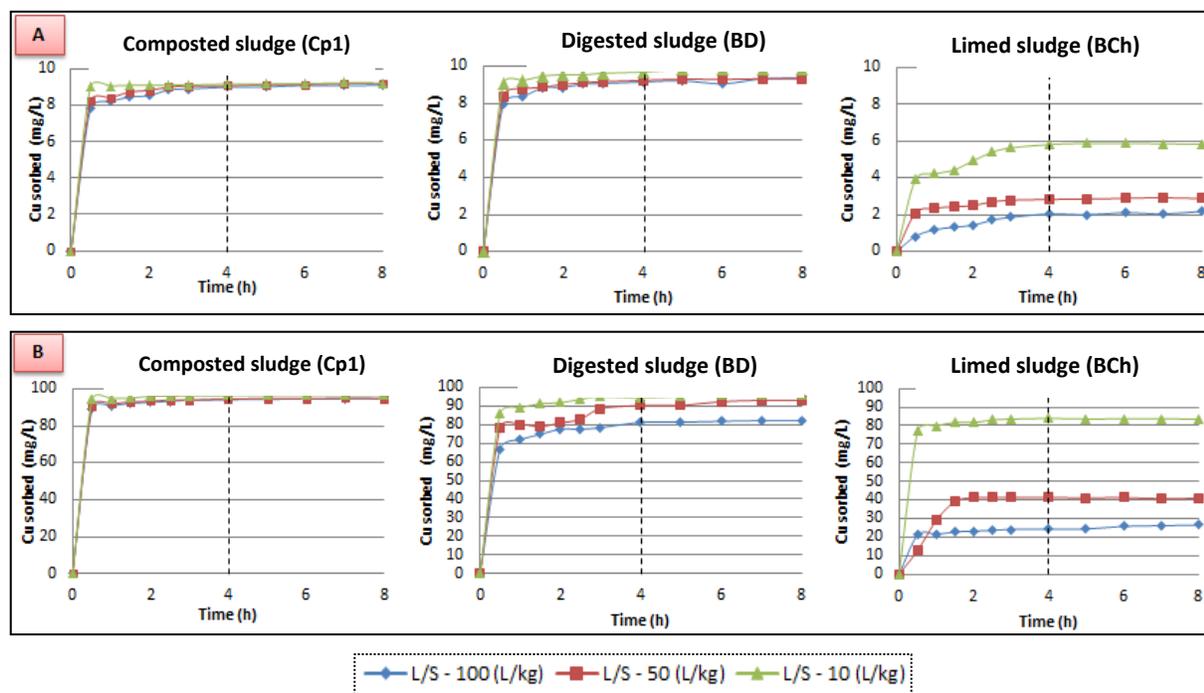


Figure 49: Kinetic adsorption of Cu on Cp1, BD and BCh sludge at different initial Cu concentration in adsorption solution: A – 10 mg/L and B – 100 mg/L and different L/S ratios.

The kinetic of Cu biosorption by different kinds of sludge for the two initial Cu concentrations presented a shape characterized by:

- A strong increase of the Cu biosorbed concentration at equilibrium during the first 30 min of contact solution-sludge. This indicated the high Cu sorption capacity on sludge at beginning of adsorption process.
- A very slow increase of Cu sorption until reaching a state equilibrium after 4h.

Therefore, the necessary time to reach the equilibrium is 4h and an increase of removal time to 8h did not show any notable effect.

The Cu sorption on granular sludge is described in the literature. This rapid biosorption phenomenon was consistent with the results reported by (Gai et al., 2008; Wang et al., 2010a) for Cu adsorption on aerobic granules. Wang et al., (2010a) indicate that the uptake of Cu by disintegrated aerobic granules rapidly reaches equilibrium within 35 and 20 min for metal concentrations of 40 and 80 mg/L, respectively. Gai et al., (2008) indicated the time required for equilibrium was 30 min at an initial Cu concentration of 125 mg/L. Benaïssa and Elouchdi (2011b) also investigated that Cu quickly sorbed on dried activated sludge after first 30 min at Cu concentration 100 mg/L and they set up 5h for the necessary time of Cu adsorption study.

Hence, the adsorption of Cu on sludge almost occurs quickly at the short time beginning of adsorption process for three studied sludge, followed by the slow adsorption status then the stable at equilibrium after 4 hours.

It also can be seen that the initial Cu concentrations have significant impact to the ability of Cu adsorption on sludge. When Cu concentration increased from 10 mg/l to 100 mg/l, the Cu adsorbed concentration on the sludge also went up. Hence, a higher initial concentration of Cu will enhance the biosorption process (Gulnaz et al., 2006). At low initial Cu concentrations, such as 10 mg/L, it could be hypothesis that all Cu ions were biosorbed onto binding sites on sludge surfaces. Then Cu sorption capacity increased at higher initial Cu ions concentration (incase of 100 mgCu/L). This result could be attributed to an increase of binding or interaction between Cu ion and active sites on sludge surface (Yunus Pamukoglu and Kargi, 2006b). The amount of Cu sorbed on sludge depends on the occupation of activate sites on the sludge surfaces (Ho, 2006).

Adsorbent concentration is a significant factor impacting adsorption processes, determining the adsorbent – adsorbate equilibrium of the system (Deveci and Kar, 2013). This notion was reflected by the liquid to solid ratio (L/S). From the literature reviews, the adsorption studies of heavy metal on sludge were often set up at L/S ratio of 50 (L/kg), this ratio was also very close to stabilized sludge concentration in WWTPs (20 gTS/L). In present study, we established the Cu adsorption experiments at three L/S ratio of 100, 50, and 10 (L/kg). Normally, the adsorption of metals ions is due to the copper interactions with the reactive functional groups of the biosorbents (Sadeek et al., 2015). In case of composted sludge, when L/S ratio decrease from 100 (L/kg) to 10 (L/kg), the Cu adsorbed concentration increase from 8.97 mg/L to 9.15 after 4h of adsorption. The similar trends were also obtained for BD and BCh sludge in the same condition of experiment. Apparently, the decrease of the L/S ratio from 100 to 10 (L/kg) resulted in an increase of external surface of sludge particles, yielding more binding sites for Cu ions, and therefore, more efficient biosorption (Yunus Pamukoglu and Kargi, 2006c).

The sludge physical-chemical structures and characteristics also influence the Cu adsorption process. Comparing Cp1 sludge and BD sludge, similar Cu adsorption capacity was seen at 10 mg/L, and very small difference was obtained at 100 mg/L of initial Cu concentration. For details, Cu adsorbed concentration was around 95 mg/L for BD sludge and approximated 96mg/L for Cp1 sludge at 100 mg/L initial Cu concentration, when the L/S ratio was set up at 100 (L/kg).

In BCh sludge, $\text{Ca}(\text{OH})_2$ adding has an effect on Cu adsorption (Figure 49). Insoluble Cu hydroxide starts precipitating, resulting in lower amount of copper biosorbed at equilibrium (Benaïssa and Elouchdi, 2011c). In case of BCh sludge, the fluctuation of Cu adsorption was seen at L/S ratio of 100 (L/kg), the similar stable trend after reaching equilibrium from 4h to 8h was seen at lower L/S ratio of 50 and 10 (L/kg) in both of initial Cu concentration.

According to the above described results, we can conclude that the necessary time to reach the equilibrium for all sludge samples is 4h. A L/S ratio 50 (L/kg) was proven to be adapted for our experiments. These conditions will be used for investigating Cu adsorption onto all kinds of conventional and granular sludge.



II.3.2. Copper adsorption isotherm on different kind of sludge

Adsorption is usually described through isotherms. In this present study, the Cu biosorption isotherm (q_e vs. C_e) obtained for the different sludge samples under a L/S ratio of 50 (L/kg) and Cu ion concentration in adsorption solution ranging from 0 to 1000 mg/L (part 2 - III.2.2, page 108), are reported in Figure 50.

In all cases the shape of the isotherm corresponds to 'L' type. 'L' type is one of isotherm classes for liquid-solid adsorption (Limousin et al., 2007). This type indicates the relationship between the Cu ions concentration equilibrium remaining in solution (C_e) and Cu adsorbed amount on the sludge (q_e) following a concave curve. Cu ion adsorption isotherm on sludge did not reach any plateau very clearly. From the plot of this isotherm, the curves were devised in two main regions:

- The linear region, where the steep slope of the beginning portion of the Cu-sludge isotherm indicated a very high affinity at the lower metal concentration. In this region of adsorption curve, the Cu adsorption ability onto sludge were illustrated by distribution efficient (K_d) following linear isotherm model;
- The concave region at the end of adsorption curves, in which, the concentration of Cu in solution increased but the Cu adsorption on sludge presented the downward trend. The decrease of Cu adsorption ability onto sludge may be a reflection of complexation or saturation indicating that once this reached value no further adsorption occurs.

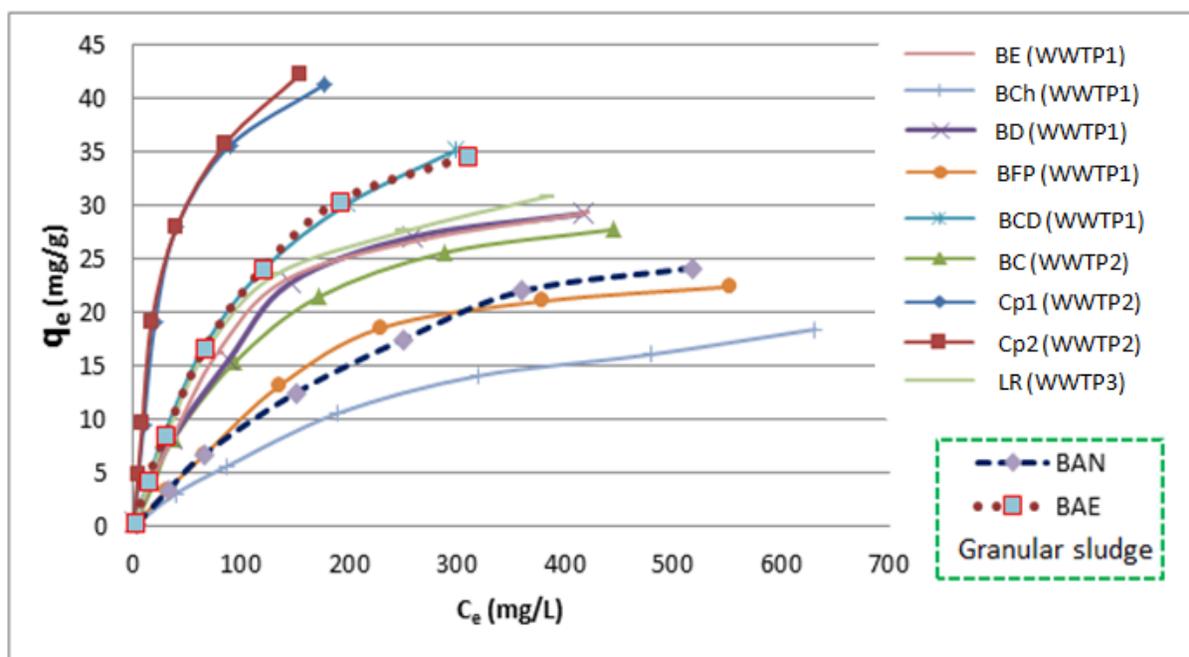


Figure 50 : Isotherm of Cu biosorption by different sludge at experiment adsorption conditions



Therefore, in order to study sludge copper adsorption availabilities of different sludge, it is important to establish the most appropriate correlation for the equilibrium curve. Equilibrium data, commonly known as adsorption isotherms, are basic requirements for the design of adsorption systems. Considering the shape of the isotherms, three models were used for their description: first linear isotherm model for the very low initial Cu concentration (from 0 mg/L to 400 mg/L), Freundlich for the intermediate concentrations and Langmuir (even though plate was never reached). These models have been described in the material and methods part (part 2 - III.2.2, page 108).

The results of the different modeling scenario are summarized in Table 28. These modeling allowed us to determine the capacity of Cu bio-sorption onto sludge and the Cu distribution between the liquid phase and the sludge particulate phases.

When the experimental data was applied to adsorption models, the quality model which fitting to the experimental data is typically assessed based on R^2 values. Closed to unity values are deemed to provide the best fit. From the results obtained, it appeared that the linear model can fit the first part of the isotherms. Freundlich and Langmuir models led to acceptable fits of the experimental results over the experimental range with good regression coefficients

- **Linear isotherm model**

The distribution coefficient (K_d) of linear isotherm model described the Cu distribution capacity between the sludge particulate phase and soluble phase after adsorption. Thus, high K_d values illustrate strong sorption and retention of Cu onto sludge solid phase. According to the results obtained in Table 28, all sludge samples exhibited different Cu sorption capacity.

The K_d values associated to conventional sludge samples varied according to the following decreasing order: Cp1 > Cp2 > BCD > LR > BD \approx BE > BC > BFP > BCh. The lowest K_d values were obtained in BCh and BFP sludge and equal to 0.098 ± 0.022 (L/g) and 0.142 ± 0.023 (L/g) respectively. It could be hypothesis that the components in the soluble phase of these sludges have strong affinity for Cu, which reduced the Cu sorption and distribution on particulate phase of sludge.

In case of granular sludge, the K_d of BAE was higher than the K_d value of BAN. The K_d of BAE was equal to 0.431 ± 0.093 (L/g), while the K_d value of BAN was equal to 0.134 ± 0.026 (L/g). Compared to conventional sludge, the K_d values of BAE granules was comparable the K_d values of BCD sludge, whereas the K_d values of BAN sludge was in the range of K_d measured for BCH sludge and BFP sludge.



Table 28: Constants and correlation coefficient for Linear, Langmuir’s and Freundlich’s equilibrium adsorption equation for Cu at a L/S ratio of 50 L/kg

Sludge		Langmuir model			Freundlich model			Linear model	
		q_{max} (mg/g)	K_L (L/mg)	R^2	K_f (L/g)	1/n	R^2	K_d (L/g)	R^2
Conventional activated sludge	BE (WWTP1)	39.06 ± 8.63	0.085 ± 0.036	0.98	0.318 ± 0.051	0.866 ± 0.031	0.98	0.323 ± 0.058	0.99
	BCh (WWTP1)	26.80 ± 4.53	0.038 ± 0.017	0.99	0.280 ± 0.013	0.896 ± 0.035	0.99	0.098 ± 0.022	0.99
	BD (WWTP1)	39.52 ± 5.78	0.089 ± 0.018	0.98	0.916 ± 0.433	0.845 ± 0.028	0.99	0.324 ± 0.077	0.97
	BFP (WWTP1)	34.60 ± 6.72	0.095 ± 0.012	0.98	0.982 ± 0.023	0.772 ± 0.128	0.99	0.142 ± 0.023	0.98
	BCD (WWTP1)	53.47 ± 4.81	0.191 ± 0.019	0.98	1.674 ± 0.078	0.636 ± 0.078	0.99	0.901 ± 0.074	0.99
	BC (WWTP2)	37.59 ± 0.54	0.078 ± 0.031	0.97	0.307 ± 0.043	0.886 ± 0.037	0.97	0.277 ± 0.059	0.97
	Cp1 (WWTP2)	50.50 ± 3.77	0.184 ± 0.036	0.99	1.479 ± 0.083	0.746 ± 0.012	0.99	1.438 ± 0.241	0.98
	Cp2 (WWTP2)	50.76 ± 2.79	0.186 ± 0.015	0.98	1.408 ± 0.619	0.742 ± 0.025	0.99	1.743 ± 0.300	0.97
	LR (WWTP3)	40.16 ± 3.96	0.129 ± 0.029	0.98	1.012 ± 0.093	0.867 ± 0.025	0.98	0.383 ± 0.072	0.99
Granular sludge	BAN	42.19 ± 0.48	0.132 ± 0.017	0.97	0.937 ± 0.039	0.841 ± 0.211	0.99	0.134 ± 0.026	0.98
	BAE	49.65 ± 1.11	0.141 ± 0.016	0.97	0.972 ± 0.162	0.825 ± 0.184	0.97	0.731 ± 0.093	0.99



Effect of surface functional groups of sludge characteristic on Cu adsorption ability

In fact, the sludge surface is dominated by permanent of functional group on the sludge particulate fraction and therefore has the capacity to sorb relatively large amounts of metal ions (Laurent et al., 2010). In this study, acid-base titration and infrared (FTIR) spectra method (part 2-II.3.3; page 98) were complexed to determine how many types of functional groups were involved in the Cu adsorption and distribution on sludge particulate and soluble phases. The pKa values of functional groups present on sludge particulate and soluble, were investigated via titration acid-base (part 2-II.3.1, page 95) fitting PROTOFIT software in respect with IR spectra. Types of sites associated with their pKa for sludge particulate and soluble phases were showed in Figure 51.

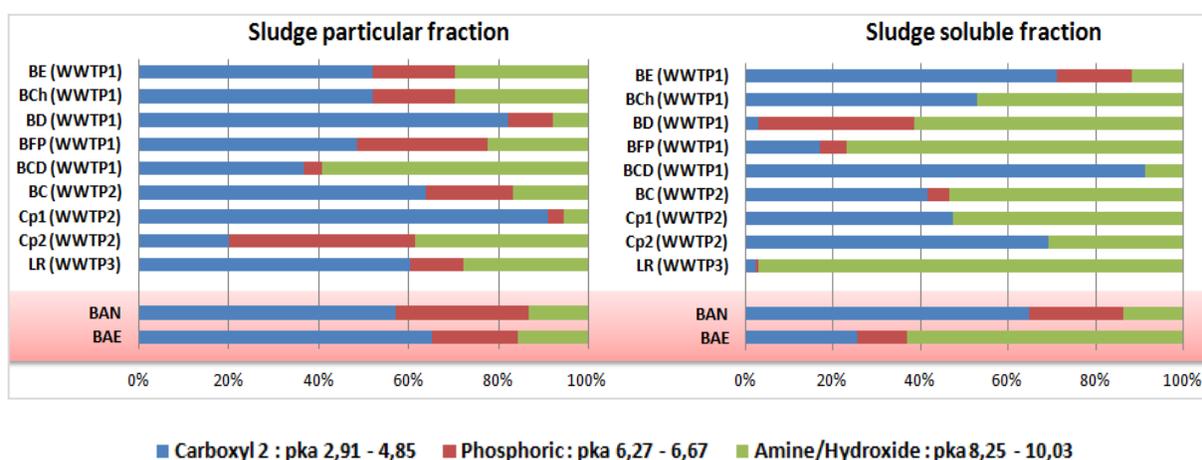


Figure 51: The distribution of functional groups according to pKa values of acid-base titration method for conventional and granular sludge.

Comparing between conventional sludge, the highest percentage of carboxyl groups and phosphoric groups was found in particulate fraction of Cp1 and Cp2, respectively. These high percentages are related to high K_d values (high Cu adsorption on sludge particulate fraction). According to (Laurent et al., 2009), carboxyl and phosphoric groups had significant effect on metal ions adsorption due to complexation and precipitation mechanisms.

Wang et al., (2010a) illustrated the key binding site of carboxyl group on aerobic granular sludge for the cationic Cu and their results were in accordance with those obtained in Figure 51 for BAE. Aerobic granular sludge contained high percentages of carboxyl groups in particulate fraction with 64.8%, but only 25% in soluble fraction. The different distribution of carboxyl group between particulate and soluble phase on aerobic granular sludge can be involved in more Cu affinity for particulate phase thus explaining high K_d value from linear isotherm (Table 28).

- **The Freundlich isotherm model**

In this model, the $1/n$ values are representative of the shape of the isotherms. The $1/n$ values were relatively comparable in different type of sludge, indicating that the shapes of the curves were closed. The $1/n$ value in Freundlich equation ranged from 0.636 ± 0.078 to 0.986 ± 0.035 (Table 28). The situation $1/n < 1$ or $n > 1$ is most common, and may be due to a different distribution of surface sites or any factor that causes a decrease in adsorbent-adsorbate interaction with increasing surface density (Site, 2001). The values of n within the range of 1 – 10 (or $1/n$ in range of 0.1 – 1.0) represent good adsorption. In the present study, $1/n$ ranges between 0.1 and 1.0, thus indicating good physical adsorption of Cu ion onto all kind of sludge.

The constant K_f of Freundlich isotherm model allows estimating the adsorption coefficient of Cu on sludge. For conventional sludge, K_f values ranged from 0.280 ± 0.013 to 1.674 ± 0.078 (L/g). The sample corresponding to K_f increasing values were: BCh < BC < BE < BD < BFP < LR < Cp2 < Cp1 < BCD. Increasing K_f values corresponded to increasing adsorption ability. This result showed that sludge stabilization treatment impacted on Cu affinity. The affinity was all the more increased as the organic matter was humidified (composted sludge and reed bed sludge) and dried (centri-dried sludge).

According to literature, Freundlich isotherm is suitable to explain metal ions adsorption at high concentration while linear isotherm is often used for a narrow range of concentration or very low concentration of metal ions. The differences between the K_d and K_f values could be indicators of the quantity of adsorption sites on sludge. It could be hypothesis that, if K_d and K_f are closed, the sludge can have high adsorption capacity. In the case of Cp2 and Cp1 the K_d (438 ± 0.241 (L/g)) and K_f (1.479 ± 0.083 (L/g)) were very closed. This indicated that, according to this range of Cu concentration in this study (from 0 to 1000 mg/L), Cu can adsorb in all sites on the surface of sludge (Barakat, 2011). There were not or small limitation of adsorption sites on these kinds of sludge. It also explains the almost straight line on the isotherm curve for composted sludge.

In some other cases, such as BFP or BCh sludge, the K_f was about 10 times higher than K_d values. These sludges showed concave isotherm curve for Cu adsorption. Thus, the potential for Cu adsorption decrease when the Cu concentration increases in solution. In addition, when copper adsorption on BCh, the $1/n$ values is always higher than other sludge, this illustrated the lower number binding sites for Cu adsorption in these sludge, and reduce the retention of Cu on particulate phase of these sludge.

K_f values for aerobic and anaerobic granular sludge were comparable. The K_f values were respectively equal to 0.972 ± 0.162 (L/g) and 0.937 ± 0.039 (L/g) for BAE and BAN granular sludge. In comparison to conventional sludge, these values stayed in the range of the K_f values between BD and BFP.

Effect of proton exchange capacity of sludge characteristic on Cu adsorption ability

In theory, the Freundlich isotherm constants (K_f) related to the biomass capacity of Cu adsorption. Therefore, the sludge might have a heterogeneous surface with various metal binding sites of functional groups rather than a homogenous monolayer. According to acid-base titration method, it is assumed that



adsorption is due to proton interaction with negatively charged organic acid functional groups on the bacterial walls or the extracellular polymers. The PEC values of sludge particulate were calculated and showed in Figure 52.

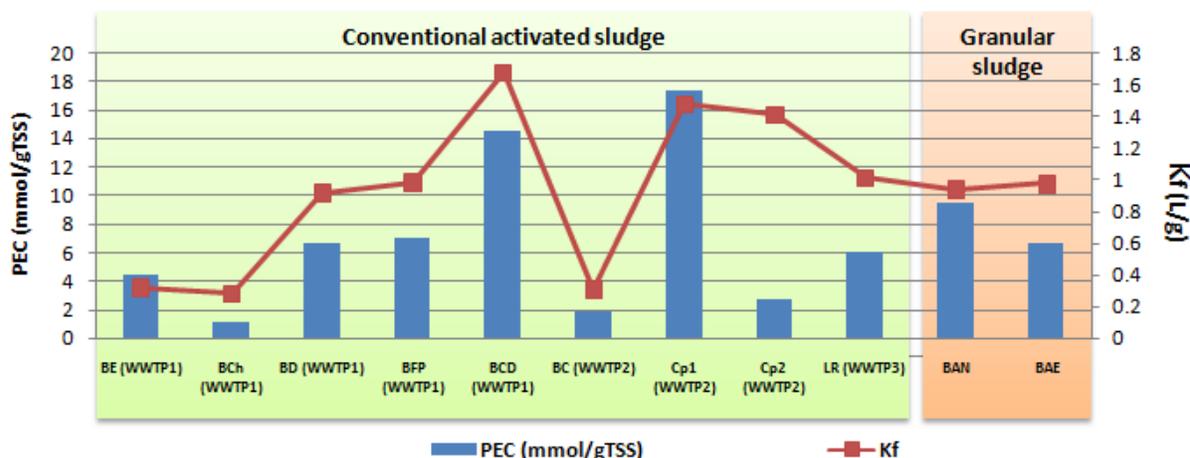


Figure 52 : Total number of ionizable functional groups on sludge particular phase

The order of PEC values could be directly linked to the Cu adsorption capacity on sludge. Indeed, high PEC values also indicated strong Cu adsorption ability (example of Cp1 and BCD).

The increase in the global Freundlich isotherm parameter (K_f) for Cu (BCH < BC < BE < BD < BFP < LR < Cp2 < Cp1 < BCD) could be explained by the increase of sludge specific surface area after composting, centrifuging, and digesting. After these stabilization processes, the sludge size decrease and enhanced proton exchange capacity which allowed a better availability of metal binding sites at the surface of sludge (Wang et al., 2010a).

Granular sludge had PEC values lower than BCD and higher than BD sludge (Figure 52). The granular sludge structure (numerous proton exchange sites, compact, dense and round structure) could favor the interaction between Cu ion in solution and active sites on surface of granules (Ho, 2006). Furthermore, during the anaerobic granulation, methanogenic process produced gas, which facilitated the formation of a more porous structure and large surface of the granules, thus increasing the exchangeable sites for binding with metal ions.

Hence, as already mentioned by (Laurent et al., 2011a; Zhou et al., 2013a), PEC values could be assumed as important factors explaining Cu biosorption efficiency on conventional and granular sludge.

- **The Langmuir isotherm model**

The Langmuir isotherm assumes monolayer adsorption onto a surface containing a finite number of adsorption sites of uniform strategies with no transmigration of adsorbate in plane surface (Limousin et al., 2007). Once a



site filled, no further sorption can take place at that site. This indicates that the surface reaches a saturation point where the maximum adsorption of the surface will be achieved. In the Langmuir model, q_{\max} represented the maximal adsorption capacity and K_L showed the affinity for the Cu.

In this study, a plate was never reached in the isotherms, so the parameters deriving from Langmuir modeling should be cautiously considered. The numeric values cannot be considered as representative of real physical mechanisms, but can give some information when comparing one sample to another.

In cases of conventional sludge, the highest values of q_{\max} was obtained in BCD with 53.47 ± 4.81 (mg/g), followed by Cp2 and Cp1 with 50.76 ± 2.79 and 50.50 ± 3.77 (mg/g), respectively. The order of q_{\max} values was as followed: BCh < BFP < BC < BE \approx BD < LR \approx Cp1 < Cp2 < BCD. This order is quite comparable to those observed with the Freundlich model: BCh and BFP had the lowest sorption ability (probably due to excessive positive charge compared to other sludge samples).

K_L provides an indication of the affinity of the metal for binding sites on the biosorbent (Pamukoglu and Kargi, 2007). BCh showed the lowest maximal Cu adsorption ability value at 26.80 ± 4.53 (mg/g) with a value of K_L of 0.038 ± 0.017 (L/mg). The order of K_L was the following: BCh < BC < BE \approx BD < BFP < LR < Cp2 \approx Cp1 \approx BCD. K_L is completely correlated to q_{\max} values, meaning that sorption were due to affinity of copper for the whole sludge.

Anaerobic and aerobic granular sludge showed maximal Cu adsorption value lower than composted sludge and centrifuged sludge but higher than others sludge. The q_{\max} values obtained 42.19 ± 0.48 and 49.65 ± 1.11 (mg/g) for anaerobic and aerobic granules respectively were comparable.

Effect of organic matter and cation exchange capacity on Cu adsorption ability

It is difficult to correlate biochemical composition and q_{\max} values. As demonstrated in previous studies, the cation exchange capacity is a decisive parameter in trace element heavy metals sorption behavior description. Indeed, metal adsorb onto the sludge by cations exchange. Consequently, CEC is directly related to the capacity of the sludge to adsorb heavy metals. The greater CEC value, the more exchange sites on the sludge minerals will be available for metal retention (Lu and Xu, 2009). Therefore CEC could be considered as an important factor controlling heavy metals adsorption in sludge matrix.

Cation exchange capacity (CEC) values of conventional sludge and granular sludge were plotted in Figure 53, The CEC values of sludge obtained high values. When sludge was applied to soils, they are supposed to increase the cation exchange capacity of soils. This point is of interest especially for sandy and loamy soils that lack clay and thus have poor cation binding capacity. It is known that organic matter behave partially as amphoteric substance with the negative charge attracting cations and hence increasing the cation exchange capacity of sludge. In this relationship, granular sludge (BAN/BAE) and some conventional sludge such as BCD, Cp2, Cp1 and BC presented higher CEC values than other sludge.



During the wastewater treatment which contained high heavy metal concentration, while cation exchange capacity of sludge often affect to the adsorption ability of metal ion on sludge by cation exchange mechanisms, dissolved organic carbon (DOC) impacts to metal ion distribution between soluble and particulate fractions in sludge and/or mobilization during leaching process.

Organic matter (OM) in sludge has an important role for metal adsorption capacity (Liu et al., 2014). OM availability and selectivity for metal depends on the specific electrochemical and structural properties of the ligand and metal ions. Cu generally reacts with OM by forming stable coordination complexes rather than by forming compounds via hydrogen bonding or electrostatic attraction because of its low hydration energy and low charge (Liu et al., 2014). Previous studies demonstrated the high affinity of Cu for OM, thus leading to strongly adsorption of Cu on sludge matrix.

Therefore, the amount of sludge organic matter could be also considered as an important parameter driving Cu adsorption ability on sludge.

The effect of sludge organic matter (OM) and CEC parameters on Cu adsorption ability were presented in Figure 53.

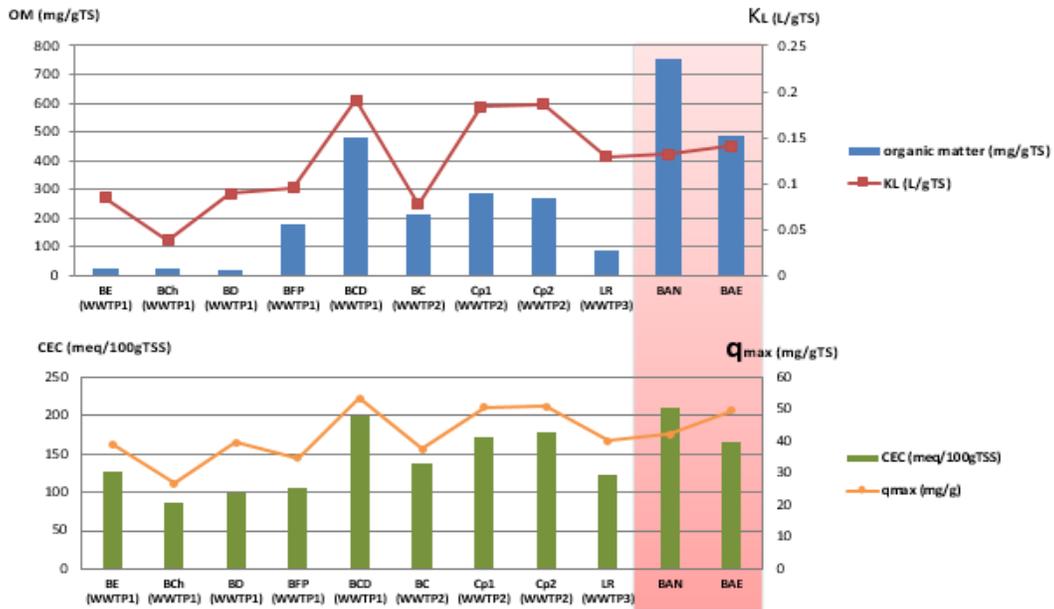


Figure 53: Relationship between K_L and organic matter (OM), relation between q_{max} values (Langmuir model) and cation exchange capacity (CEC) values of native sludge matrix.

Figure 53 showed that the OM content and CEC values could be put in relation with K_L and q_{max} values evolution. Some conventional sludge such as Cp2, Cp1, BCD and granular sludge (BAN/BAE) contained higher amount of organic matter and capacity of higher cation exchange capacity compare to other sludge samples. These sludges also presented high affinity and adsorption capacity of Cu onto sludge matrix following the constants of Langmuir isotherm model. Limed sludge and thickened sludge present low OM



and CEC values which were related to low Cu sorption on sludge particulates phases. The low organic content of the soluble phase could partly explained lower Cu affinity for limed sludge.

More interestingly, BFP presented organic matter concentration values comparable to BC, and CEC comparable BD. However, BFP sludge exhibited Cu adsorption capacity lower than BC and BD sludge (see q_{max} values on Table 28 of Langmuir isotherm model).

The reasons for the low copper adsorption affinity and capacity in the cases of BFP and BCh sludge can be explained by cations adding by lime (on limed sludge) together positive polymeric substances during sludge polymeric flocculation and/or coagulation during filter-pressed stabilization. Those positively charged element could compete with Cu ion and reduced Cu adsorption on these sludge.

Granular sludge is known as an organic rich matrix and composed by microorganism, EPS and mineral matter. The biochemical compositions of these organic matrices are able to make interaction and binding affinity with Cu ion in aqueous solution (Wang et al., 2010a). Thus, high organic matter and cation exchange capacity can explain high Cu biosorption efficiency on aerobic granules in the context of this study.

Effect of biochemical composition of sludge on Cu adsorption ability

Sludge are made of different biochemical compounds such as proteins, humic-like acid (or humic-like substances) and polysaccharides. These compositions were evaluated on both of sludge total and soluble phases (see chapter 1). The proportion of biochemical compositions in soluble and total phase of sludge matrix could be reflected the Cu adsorption capacity on sludge. The distribution of these components was presented in Figure 54.

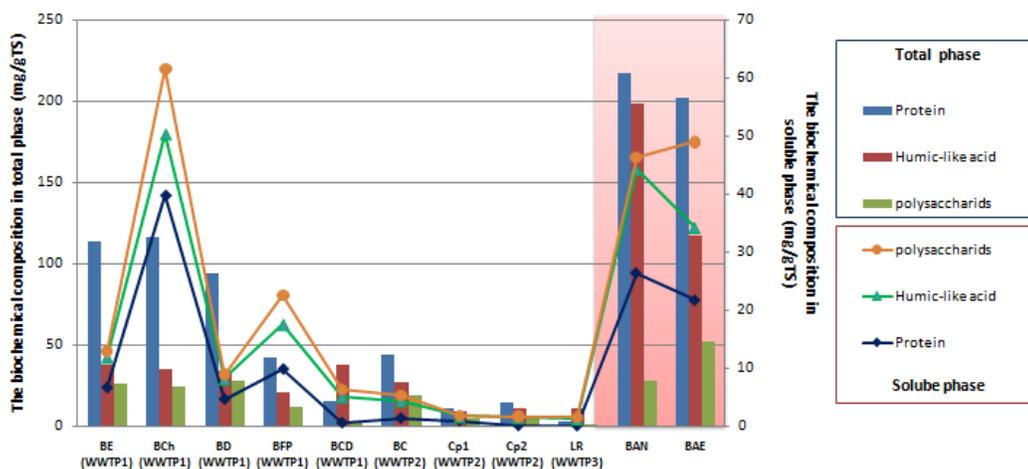


Figure 54 : The biochemical compositions distribution in soluble and total phases of sludge matrix.

The presented results show that the treatments of the sludge (in particulate liming or filter-press) have a notable impact on the biochemical composition of the sludge and also on the nature of the biochemical



species of the soluble phase. Whatever the origin of the sludge, the total sludge consists mainly of protein and humic substances and to a lower extent of polysaccharide. However, the soluble fraction of the different sludge samples is composed mainly of polysaccharide and humic substances and their concentrations in both soluble and total phase increased after treatment like liming, thickening and granulation with anaerobic/aerobic process. Moreover, Cp2, Cp1, LR and BCD contain low biochemical components in both total and soluble phase, compared to other conventional sludge and granular sludge.

Figure 54 showed that the biochemical composition of the soluble may influence the metal ion adsorption capacity. Biochemical composition led to different proportion of functional groups in the soluble and particulate organic matter such as carboxyl, phosphoric, amine and hydroxyl groups, which are considered as effective adsorbent for metal ion (Wei et al., 2016).

However, high concentration of biochemical components in sludge matrix is not directly linked with metal sorption enhancement. For example, limed sludge contained the highest concentration of biochemical species in total sludge but showed the lowest maximum Cu adsorption capacity according Langmuir and Freundlich isotherm models. The opposite trend was seen in Cp1, Cp2, LR and BCD sludge. Compared to Cp1, Cp2 and BCD sludge, granular sludge also had high biochemical components concentration in total sludge but low Cu adsorption capacity.

One hypothesis is that this phenomenon can be explained by the distribution of biochemical components between soluble and total phase (Figure 55).

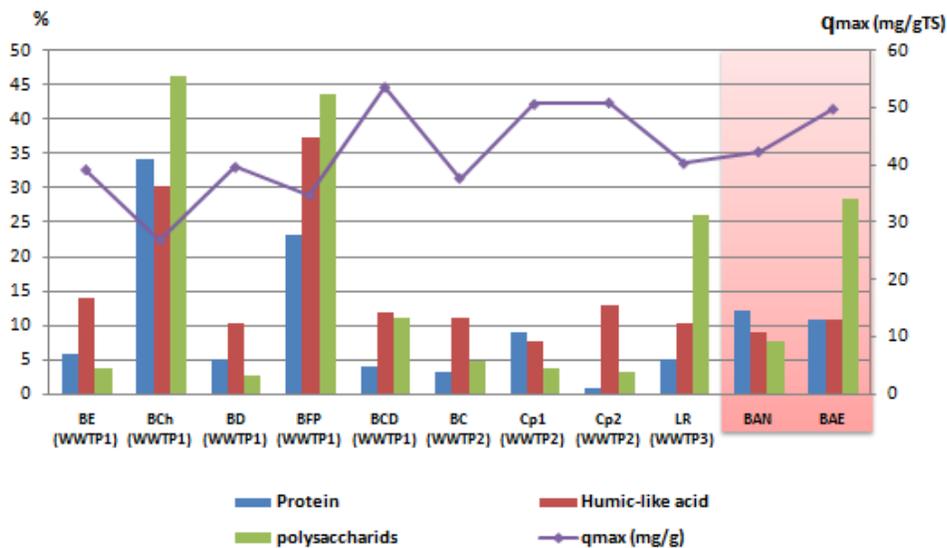


Figure 55: The correlation between the maximum Cu adsorption capacity and percentages of biochemical composition distributed in soluble phases of sludge.



Following Figure 55, more important biochemical components concentration in soluble phase could be closely linked to q_{\max} . Low q_{\max} values for BCh and BFP can be linked to high biochemical components content in soluble phase.

II.4. Modeling Cu sorption on grass and paddy soil samples

The most important process affecting the behaviors of heavy metals in soil is the adsorption of metals from liquid phase into the solid phase. The adsorbents used in this part study consisted of: grass soil (from France) and paddy soil (from Vietnam) as introduced in part 2 of material and method part (See III.1.2, page 104)

The batch method was used to measure the copper adsorption ability on soils. The test process was completed with initial concentrations of solutions ranging from 0 to 1000 mg/L (Part 2-III.2.2, page 108). After determining the equilibrium time, the adsorption isotherms were modeled by the linear, Langmuir and Freundlich isotherm models. The L/S ratio at 10 (L/Kg) is chosen for studying soils sorption/desorption abilities.

The time required for Cu adsorption on soils is an important step and was determined firstly. Batch experiments were conducted to explore the Cu adsorption capacity by grass soil and paddy soil at the initial Cu concentration of 100 mg/L.

The relationship between the Cu concentration and amount of Cu adsorption on soil at equilibrium was presented in Figure 56. Base on the shape of isotherm adsorption curve, we can be recognized two main regions according to the relationship between the equilibrium Cu concentration in solution and the amount of Cu sorbed on solid soil at different L/S ratios. At low initial concentration, in range of 0– 400 mg/L, the curve of (C_e vs q_e) were linear and then changed to concave curve when Cu concentration in solution increased.

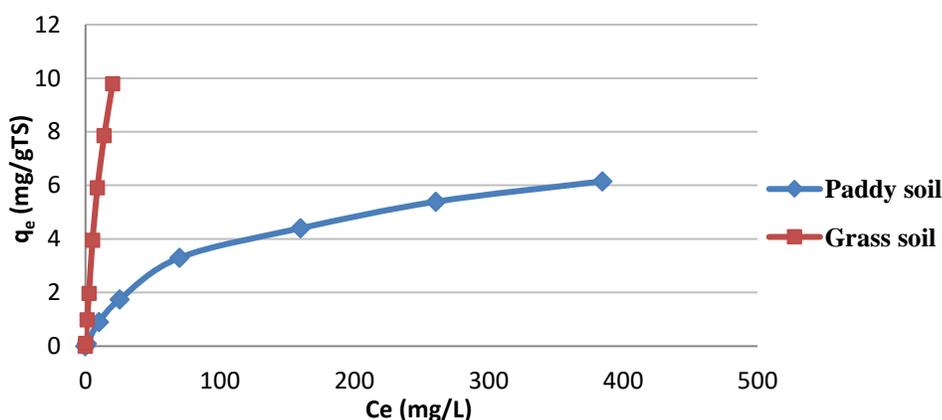


Figure 56 : Cu adsorption isotherm on grass soil and paddy soil

The Linear, Freundlich and Langmuir equations were used to fit the adsorption data. The fitting results showed that these two adsorption equation can mathematically fit the data well. Almost the correlation



coefficient are greater than 0.98. As no plate was reached, the Langmuir model values as to be cautiously interpreted, especially in case of grass soil.

Table 29 : Results of isotherms models for Copper adsorption on soils at L/S ratio of 10 L/kg.

Soils	Langmuir models			Freundlich model			Linear model	
	q_{\max} (mg/g)	K_L (L/mg)	R^2	K_f (L/g)	$1/n$	R^2	K_d (L/g)	R^2
Grass soil	25.32 ± 1.45	0.033 ± 0.002	0.99	0.748 ± 0.115	0.895 ± 0.076	0.99	0.752 ± 0.143	0.98
Paddy soil	7.24 ± 0.78	0.012 ± 0.001	0.98	0.135 ± 0.003	0.691 ± 0.025	0.98	0.068 ± 0.009	0.97

Linear model: The K_d value was 10 time lower for paddy soil compare to grass soil.

The Langmuir model: The change in the trends of the maximum adsorption of copper in the soils is depending clearly on the kind of soils. The copper adsorption capacities on grass soil are higher than those on paddy soil. Both of q_{\max} and K_d values of Cu adsorption on grass soil were always higher than that of Cu adsorption on paddy soil.

The Freundlich model: as for the other model kinetics parameters of Freundlich model were higher for grass soil, compare to paddy soil.

These phenomena could be understood based on the higher clay and organic matter which often had attractive for Cu adsorption in grass soil than in paddy soil. These results provide evidence to the interpretation that the kind of soil is important factor influencing the adsorption of copper ion

II.5. Modeling copper sorption isotherms in sludge amended soils: case of digested sludge on grass and paddy soils.

In most of the studies, the interactions between soil and HM are investigated by adding a doping solution of the concerned metal. The investigations of part II.4 and II.5 were compulsory to allow the description of the sorption of Cu on the two different matrix of interest: sludge for one part (II.4) and soil for another part (II.5).

The approach is an important step of our methodology (understanding interaction between sludge/soil and copper) but is far from representing the reality of landspreading. During landspreading, HM is brought to the soil through sludge application. It is thus very important to implement batch tests mixing soil and sludge.

Digested sludge sample (BD) was amended on both of grass soil and paddy soil. The batch adsorption tests were conducted at three sludge application rates (tons of dried sludge/(ha.year): 3, 30 and 150 tons sludge/(ha.year)).



All the adsorption conditions were repeated on desorption experiments (see in part 2 - III.2.2, page 108). Each test was duplicated. The Cu adsorption isotherm on digested sludge amended soils was presented in Figure 57:

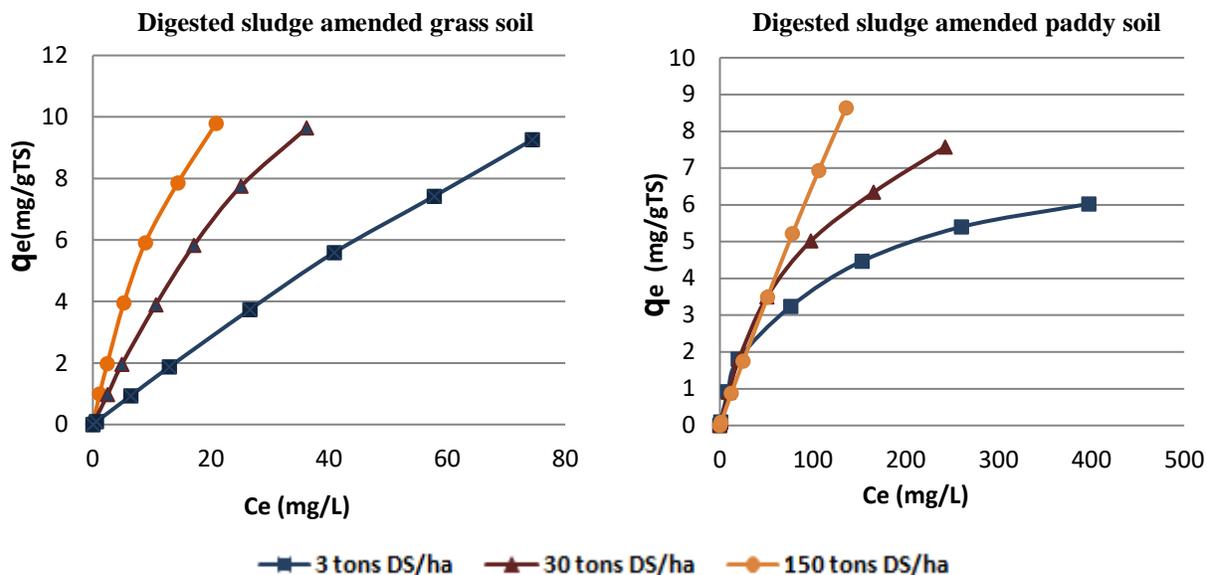


Figure 57 : Isotherm of Cu sorption on digested sludge amended grass soil and paddy soil at different application rates.

The shapes of isotherm adsorption curve presented a similar tendency for a given landspreading rate on sludge amended grass and/or paddy soil. Besides, when sludge application rate increased from 3 tons DS/(ha.year) to 150 tons DS/(ha.year), the isotherm adsorption curves also changed from concave to straight lines. Thus linear, Freundlich and Langmuir adsorption isotherm were used to determine Cu adsorption kinetics parameters. The results are presented in Table 30, the fitting of parameters mathematically obtained by linear, Langmuir and Freundlich equation, was very good.

Effect soils origin and sludge amending rate and on affinity for copper

From the results obtained in Table 30, sludge amended grass soil had higher Cu adsorption ability compare to sludge amended paddy soil at any sludge application rates.

- **Linear model:**

K_d values were around 10 times higher in grass soil compare to paddy soil whatever the landspreading rate.

Grass soil: Sludge landspreading led to a slight increase of K_d value. The increase was in accordance with the rate of sludge amending: K_d increased with sludge amending rate.

Paddy soil: K_d values are not significantly different according to the amending rate.



- **Langmuir model:**

q_{\max} value for grass soil were higher to paddy soil for a considered amending rate.

Grass soil: q_{\max} value was quite comparable for non amended and 3 to 30 (tons DS/(ha.year)) amended soils. q_{\max} increased was noticeable for an amending rate of 150 (tons DS/(ha.year)).

Paddy soil: the same tendencies were observed.

- **Freundlich model:**

K_f values were around 10 times higher in grass soil compare to paddy soil whatever the landspreading rate.

Grass soil: K_f values were quite comparable for non amended and 3 (tons DS/(ha.year)) amended soils. $1/n$ values were quite comparable for non amended and amended soils. K_f values increased with increasing amending rate. Therefore, high rate of sludge application on soil can increase the affinity for Cu adsorption on the particulate phase of soil. These results coincide with the q_{\max} values tendency at increasing sludge application rates of the Langmuir isotherm model. The K_f values of Freundlich isotherm model also correlated with K_d values of linear model.

Paddy soil: K_f values and $1/n$ values were not drastically modified whatever the amending rate.

Surprisingly, the only parameters which were really affected by sludge amending rate were q_{\max} and K_d and K_f for grass soil and only q_{\max} for paddy soil



Table 30 : Copper adsorption isotherm kinetics parameters for digested sludge amended on soils at different sludge application rates.

Digested sludge amended soil (tons DS/(ha.year))			Langmuir model			Freundlich model			Linear model	
			q_{max} (mg/g)	K_L (L/g)	R^2	K_f (L/g)	$1/n$	R^2	K_d (L/g)	R^2
Grass soil	L/S – 10 (L/kg)	0	25.32± 1.45	0.033 ± 0.002	0.99	0.748± 0.115	0.895± 0.076	0.99	0.752± 0.143	0.98
		3	20.41 ± 1.49	0.031 ± 0.009	0.98	0.802 ± 0.056	0.918 ± 0.076	0.99	0.810 ± 0.033	0.99
		30	27.78 ± 1.53	0.051 ± 0.003	0.98	0.948 ± 0.037	0.916 ± 0.045	0.97	0.936 ± 0.242	0.99
		150	52.63 ± 1.27	0.054 ± 0.007	0.99	1.561 ± 0.016	0.881 ± 0.064	0.97	1.144 ± 0.089	0.99
Paddy soil	L/S – 10 (L/kg)	0	7.24± 0.78	0.012 ± 0.001	0.98	0.135± 0.003	0.691± 0.025	0.98	0.068± 0.009	0.97
		3	7.90 ± 0.11	0.015 ± 0.009	0.98	0.144 ± 0.009	0.774 ± 0.067	0.95	0.095 ± 0.006	0.99
		30	10.75 ± 0.72	0.029 ± 0.004	0.99	0.157 ± 0.006	0.698 ± 0.015	0.98	0.088 ± 0.002	0.99
		150	47.62 ± 2.09	0.030 ± 0.004	0.96	0.161 ± 0.006	0.685 ± 0.010	0.99	0.069 ± 0.002	0.99



Sludge contain high amount of organic matter and can thus enhance organic matter content in soil when they are amended. The increase of sludge application rate on soil can increase the organic matter amount; therefore increasing the negative charge of organic functional groups on the soil active sites (Bravo Vinaja and others, 2012). These groups can bind with Cu during the adsorption process and promote the Cu sorption efficiency.

Furthermore, Cu is known to have high affinity for organic matter (OM). Organic matter complex formation is recognized as one of the most effective mechanisms of Cu retention in soils, together with adsorption of CuOH^+ on clay surfaces and formation of a basic Cu carbonate from Ca carbonate (Polo et al., 1999). As expected, Cu adsorption in this work increase as the sludge dose applied to soil is higher and significant at 150 tons DS/(ha.year). So the increase of sludge content clearly affected copper ion sorption capacity on soil, especially when an important amount of OM has been added.

By analyzing adsorption isotherm parameter, it can be demonstrated conclusively that adding sludge on soil have effect to the copper adsorption. More sludge application will enhanced the copper adsorption capacity in soil, especially 150 tons DS/(ha.year). This amendment rate increases the copper availability in soil.

However, the calculation of the amount of sludge amended on soil is really deficiency if it is only based on the adsorption capacity. Other parameters such as copper desorption efficiency, mobility of copper, accumulation ability and physicochemical characteristic of soil, etc are important to choose the best sludge application rate to avoid high copper content accumulation in soil.

II.6. Description/characterization of copper desorption in sludge and soil before land spreading: Batch tests study

The mobility and availability of heavy metals are controlled by sorption and desorption characteristic of sludge and soils. The capacity of sludge and/or soils to retain and release metals is a very important factor to predict environmental impact of the use of sludge for landspreading activities. Cesar et al., (2012b) observed that sludge application on soil can mobilized or immobilized the heavy metal transportation.

The aim of this part, therefore, was to study desorption ability of copper from sludge alone, soil alone and sludge amended soil at different rates of sludge application under batch test conditions. The L/S ratio was equal to 10 (L/kg), desorption time was equal to sorption equilibrium time (4h). The tests were carried out under ambient temperature.

II.6.1. Evaluation of copper desorption ability in the different sludge samples

Copper desorption ability was assessed in batch conditions described in material and method part (part 2-III.2.3, page 110) Sludge were re-suspended in NaCl solution (0,02M), with the L/S ratio of 50 (L/kg). Figure 58 displays desorption efficiency of copper for different kind of sludge.



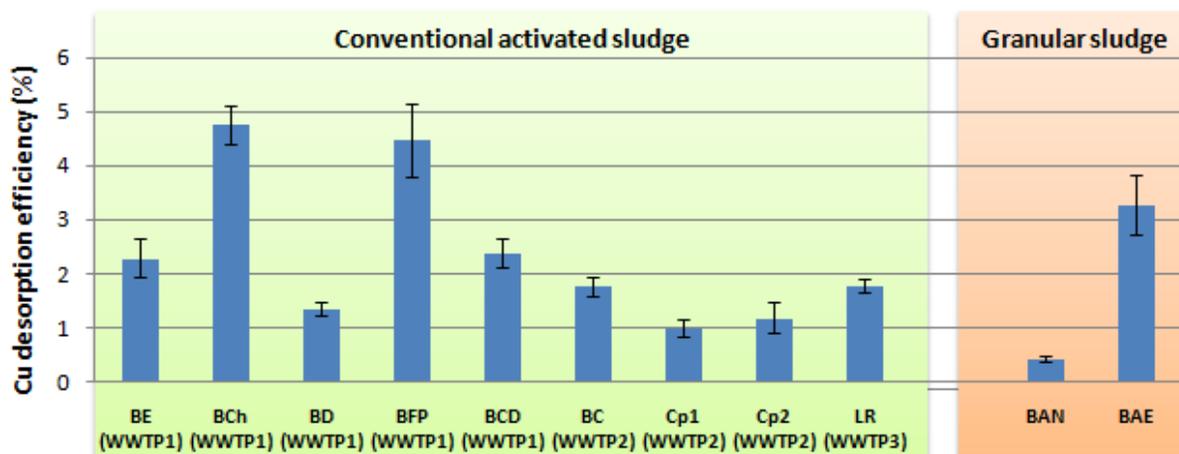


Figure 58: Percentage of copper desorption efficiency for different sludge, L/S = 50 L/kgTS

Sludge as bioorganic materials can be effectively recycled by spreading them on cultivated soils, with many beneficial effects (Tella et al., 2016). Before land application, sludge is often stabilized in order to reduce metal availability (Bravo Vinaja et al., 2012). In our study, different stabilization processes were compared for conventional activated sludge sample, and for granular sludge. According to Figure 58, all sludge samples had very low values of copper desorption percentages (< 5%).

In case of conventional sludge, the highest Cu desorption percentages was measured on BCh with $4.75 \pm 0.35\%$, followed by BFP with $4.49 \pm 0.68\%$ and other sludge. In overall, the order of Cu desorption percentages in conventional sludge were $BCh > BFP > BCD \approx BE > BC \approx LR > BD > Cp2 > Cp1$. The lowest of Cu desorption percentages obtained in Cp2 and Cp1 with $1.19 \pm 0.28\%$ and $0.19 \pm 0.06\%$, respectively. They were approximate 2 times lower than BCh. Composting and to a lower extend digestion are known to be very efficient processes for metal stabilization.

Copper desorption ability of granular sludge was very different between BAN and BAE granular sludge. BAN copper desorption percentages was only $0.43 \pm 0.06\%$, BAE copper desorption ability was $3.27 \pm 0.55\%$. This last value was comparable with those of the different conventional sludge samples. Cu desorption percentage in BAE was lower compared to BCh and BFP but higher other conventional sludge.

Desorption of copper from BAN was lower than all sludge samples. This result could be explained by the origin of the effluent (paper mill industry compare to domestic effluent for other sludge samples).

The potential relation between copper speciation and copper desorption percentages in the different sludge samples was investigated and reported on Figure 59.



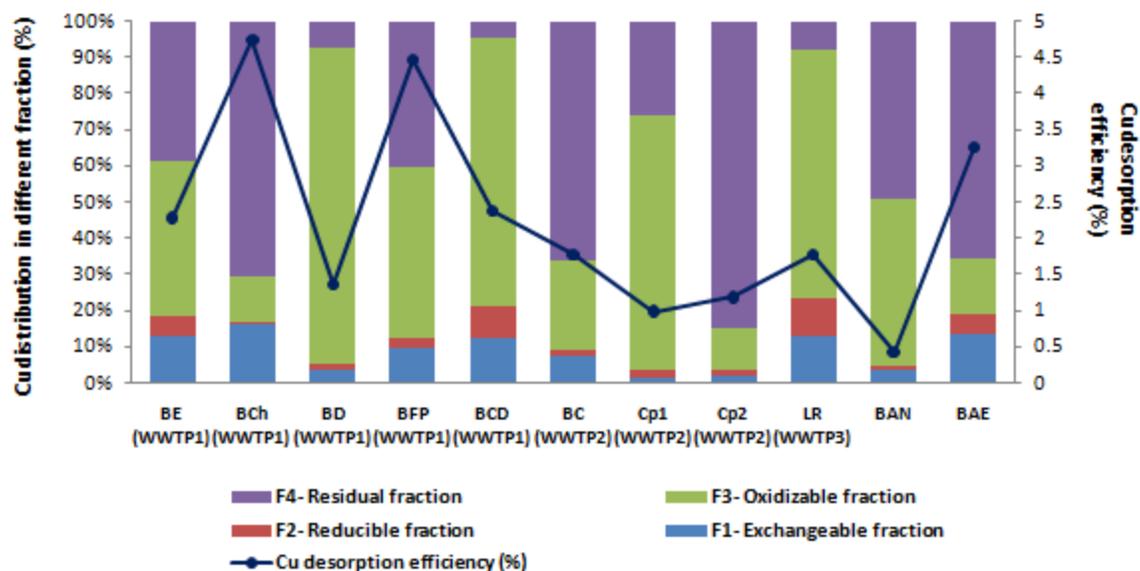


Figure 59 : Correlation between Cu desorption percentages and Cu speciation in native sludge matrix

The analysis of Figure 59 revealed, as expected, that high Cu leaching was correlate with high Cu-bound exchangeable fraction (F1). This is especially significant for BCh, BFP and BAE sludge samples. Inversely, Cp1, Cp2, BD and BAN sludge samples presented very low Cu desorption percentages corresponding to very low distribution of copper in the exchangeable fraction (F1). The exchangeable fraction (F1) provides high flexibility of metal ions that makes metals ion easily transferred from particulate phase to soluble phase (Bravo Vinaja et al, 2012). So, the exchangeable mechanism seems to a dominant process controlling copper release in this study.

Moreover, BAE created by SBR –lab scale after 47 days (Appendices 1) are characterized by an increase of EPS content during aerobic granulation. The amount of protein was much higher than those of polysaccharides in EPS components. Wei et al., (2016, 2014, 2017) indicated that PN-like substrate of EPS played an important role to bind with metals during adsorption process of granulation. The migration and enrichment of Cu in BAE can be understood as complexation with EPS components. The binding of Cu and EPS is labile and ion Cu can be replaced by ion exchange mechanism with other cation in solution (Li Wang et al., 2013). By using electrostatic desorption solution NaCl 0.02M, the copper ion in BAE can be easily replaced by Na⁺ ion with high cation exchange capacity.

II.6.2. Evaluation of copper desorption ability in soils

The potential toxicity of heavy metals in soils mainly depends on soil solid compositions, especially the amount and type of clay minerals, organic matter, and iron and manganese oxides. The bioavailability and mobility of heavy metals in soils strongly depend on their physicochemical form, chemical fraction or speciation. For all case, adsorption and desorption reactions on the surface of soils and oxides are two



important processes controlling the concentration of heavy metals in soil solution. Influence of soil characteristics on sorption and retention was also examined during these experiments.

The availability of copper in soils is also affected by desorption process, and the extent to which added copper are strongly retained against subsequent desorption. Desorption has been studied using many kinds of extracts to simulate the possible physicochemical reactions. Salt solution used popularly for metal extraction on soil. Some cations in salt solution such as Ca^{2+} , Na^+ and K^+ , etc., can replace the metals ion by ion exchange capacity for example and increase the mobility of metal during the desorption process (Xu et al., 2005). In these experiments, copper desorption in soils was tested in batch conditions in the presence of NaCl as support electrolyte mechanism at concentration of 0.02 M. at a L/S ratio of 10 (L/kg). Copper desorption results on two soils was presented on Table 31.

Table 31: Copper desorption efficiency of the two soils.

L/S ratios (L/kg)	Cu desorption efficiency (%)	
	Grass soil	Paddy soil
10	0.864 ± 0.154	12.467 ± 0.126

The results showed that the desorption efficiencies of Cu ion on paddy soil is higher than the one measured for grass soil. From soil characteristic, paddy soil presented the lower matter organic and clay contents compare to grass soil, thus explaining for high Cu mobility in paddy soil.

The Cu ions can be desorbed by un-buffered salts NaCl solution (Lachassagne et al., 2013). These results suggest that Cu adsorbed on paddy soil has relatively higher mobility because the ions adsorbed through electrostatic attraction were easily desorbed from the soils to solutions.

II.6.3. Evaluation of copper desorption ability in sludge amended soils

II.6.3.1 Effect of sludge application rates on copper desorption ability

Digested sludge sample (BD) was amended on both of grass and paddy soils. The batch desorption tests were implemented from the sludge of paragraph, suspended in NaCl (0,02M) for 4h at three sludge application rates (3, 30 and 150 tons sludge/(ha.year). The results for a L/S ratio of 10 (L/kg) are presented in Table 32.



Table 32 : Cu desorption efficiency on grass soil and paddy soil at different sludge application rates

Sludge application rates (tons DS/(ha.year))	Desorption efficiency (%)	
	Grass soil	Paddy soil
3	1.15 ± 0.17	7.40 ± 0.19
30	1.53 ± 0.18	11.10 ± 0.19
150	4.29 ± 0.16	16.98 ± 0.15

Copper desorption in paddy soil was always higher than copper desorption efficiency measured in grass soil.

Copper desorption percentage at landspreading rate of 3 and 30 (tons DS/(ha.year)) was not significantly different from non amended soil for the 2 soils: grass soil and paddy soil. Higher percentage of Cu desorption was measured in sludge amended soil compare to control soil for high doses of sludge application rate 150 (tons DS/(ha.year)).

Cu desorption percentages rose from 1.15 ± 0.17% to 1.53 ± 0.18% when increasing the sludge dose from 3 tons DS/(ha.year) to 30 tons DS/(ha.year). Cu continuously desorbed to 4.29 ± 0.16% when adding 150 tons DS/(ha.year) on grass soil, which were 3 times higher than those obtained for a sludge application rates of 3 and 30 tons DS/(ha.year). It indicated that high sludge application rates on grass soil had strong impact on copper ion mobility. It could be hypothesis that at this dose the amount of organic matter brought by the sludge impacted copper mobility.

For sludge application on paddy soil, Cu desorption percentages only extended significantly when applying more sludge at 150 tons DS/(ha.year). At lower sludge application rate such as 3 or 30 tons DS/(ha.year), the Cu desorption percentages were decreased, compared to control paddy soil. That indicated less copper ion mobility when sludge application at low rates on paddy soil.

Digested sludge were chosen to investigate the effect of sludge amending rate on copper desorption, because this stabilization process is widely used for conventional activated sludge. Surprisingly, the sludge application rate was not significantly impacted copper desorption. It could be hypothesis that the choice of BD was not the most adapted. Results from Figure 58 confirm this hypothesis as the copper desorption ability of digested sludge was very low.

II.6.3.2 Effect of sludge and soil origin on copper desorption ability

Conventional sludge and granular sludge were used to study the Cu desorption efficiency for sludge application on both grass soil and paddy soil at the rate of 3 tons DS/(ha.year) and for a L/S ratio of 10 (L/kg). The Cu desorption percentages were analyzed on soils with and without sludge application to determine the effect of sludge application to Cu mobility on soils. The results were shown on the Figure 60.



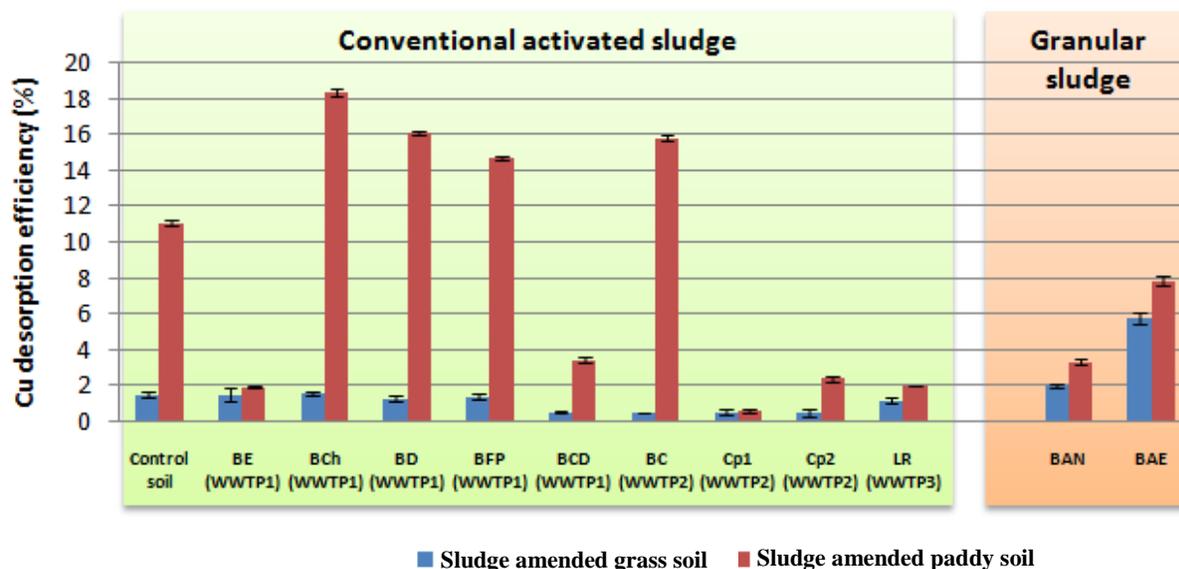


Figure 60 : The copper desorption percentages when sludge amended grass soil and paddy soil after batch test experiment.

The copper desorption efficiency is always higher in paddy soil compare to grass soil when they was added these sludge. It suggested that not only sludge characteristic effect on copper desorption but also soil physico-chemical properties.

Conventional sludge

Firstly, the order of Cu desorption on sludge amended grass soil was: BCh > BD \approx BE > BFP > LR > control-grass soil > Cp1 \approx Cp2 \approx BC \approx BCD. This result is in accordance with the Cu desorption percentages measured in conventional sludge alone (BCh > BFP > BCD \approx BE > BC \approx LR > BD > Cp2 > Cp1).

In sludge amended paddy soil the order of copper desorption percentages was: BCh > BFP > control-Paddy soil > BD \approx BC > BCD > Cp2 \approx LR > BE > Cp1. This result is not in accordance with the Cu desorption percentages measured in conventional sludge alone. At this stage it is not possible to find the reasons.

Cu desorption percentages were higher for limed sludge (BCh), and filter-press sludge (BFP) amended on grass soil and paddy soil. The copper desorption percentages were $1.59 \pm 0.13\%$ and $18.4 \pm 0.15\%$ when limed sludge amended grass soil and paddy soil, respectively. Moreover, compared to copper desorption on control soils, the Cu desorption efficiency also presented higher values when these sludge amended on both of grass and paddy soils. This result can be explained by the presence of cationic ions or polymer which out compete for sorption onto sludge negative sites.

Moreover, these sludges contain high amount of dissolved organic matter which is an important factor for the leaching of metals. The ligands contained in dissolved organic matter can bind with copper and enhanced the release of copper from solid soil to the solution. Moreover, the increase of Cu solubility is



related to the dissolution of the component such as protein, humic-like acid and polysaccharides from extracellular polymeric substances. The application of lime sludge on soil also increases the organic matter content for soils. Higher soil organic matter increase the dissolved organic matter on solution thereby promoting the formation of organic-Cu complexes and increasing Cu solubility which also increase the mobility of Cu during desorption of copper when added limed sludge in soil (Bolan et al., 2014b). Besides, the concentration of dissolved organic matter from soil is partly controlled by Ca^{2+} ions sorption which displaces Cu when adding limed sludge in soil following ion exchange mechanisms. Ca^{2+} can act as a bridge between negatively charged of dissolved organic matter and negative charge in soil particles. BCh and BFP showed a remarkable degree of extra components in solution. When these sludge applied on soils, the combination of dissolved extra polymeric substances of sludge and soils can increase the affinity for copper ion complex.

According to the literature, the pH of the soil solution often maintains at neutral to slightly alkaline condition, led to low mobility of all heavy metals. To increase the mobility of heavy metals, the pH of soil solution should be lower than 6 (Bolan et al., 2014b). The two soils used in these experiments are acidic soil with soil pH around 5 and 6 (See section - II.2, page 141). Normally, when adding lime sludge to soil, the pH of soil is supposed to be increased, thus facilitating promotion of copper stabilization in soil and reducing transfer ability to the soil solution. Comparing with initial soil, the opposite results was obtained on these experiment because copper desorption efficiency was always higher on soils amended limed sludge.

One explanation can lay in the quality of organic matter in lime sludge amended soils. Sewage sludge productions are treated with lime prior to applying on soils on the basis of 30% of dry matter. Thus, dissolved organic matter derived from sewage sludge is mainly digested to low molecular –weight organic component.

In contrast, composted sludge Cp1, Cp2 and BCD presented low copper mobility when amended to soil. Desorption efficiency were around 0.5% and 0.63% (Cp1) – 3.84% (BCD) when these sludge amended on grass soil and paddy soil respectively. This result is in accordance with the low desorption efficiencies of this kind of sludge. Thus, addition of these sewage sludges immobilized Cu ions with low copper leaching efficiency. These sludges also had high Cu adsorption and retention ability (See section II.6.1, page 162). When they are applied to soil, their sorption ability probably supported soils to stabilize Cu in particulate phase and reduce the movement of Cu ion to soluble phase.

Granular sludge:

Anaerobic and aerobic granular sludge often have very different physicochemical and biological properties due to their different composition and structure during their formation process (See part 3 - chapter 1). In these experiments, the Cu desorption efficiency showed higher values for aerobic granules amended soils compared to anaerobic granules.

In case of granular sludge amended on grass soil, both of anaerobic and aerobic granular sludge amendment increase significantly the copper desorption percentages, which were $2.03 \pm 0.13\%$ and $5.75 \pm 0.32\%$,



respectively, compared to $0.86 \pm 0.18\%$ of non-amended grass soil. The copper leaching on aerobic granular sludge amended grass soil even higher than that on all conventional sludge amendment. This indicated the increase of aerobic granular sludge copper mobility in grass soil. Similarly, the new report of (Wierzbowska et al., 2016), indicated that the application of dried granular sludge on soil changed the whole arrangement of soil structure and caused a high contamination in percolating water.

Granular sludge amended grass soil presented low copper mobility compared to control paddy soil. The copper leaching percentages from granular sludge amended paddy soil was lower than those of some conventional sludge such as BC, BCh, BFP and BD but higher than those observed for composted conventional sludge. For sandy loam-paddy soil, the organic matter and clay content were very low, sludge fertilizer is supposed to enhance the increase the compact structure, reduce the drainage and control the erosion (Balint et al., 2015). In this study, by adding granular sludge and some conventional sludge such as composted, centridried sludge may create the compact structure of soil, change the form of copper distribution and decrease the copper leaching ability.

II.6.4. Copper distribution after desorption in sludge amended soils: BCR extraction

To date, there are limited publications concerning Cu availability in granular sludge amended soils (compare to conventional activated sludge amended soils). This comparison is of great importance as the results can permit to position the choice of granular sludge process, not only in regard to its removal performances, but also in regard to the quality of the resulting sludge. So, the mechanisms of Cu desorption after granular sludge landspreading are not clearly elucidated. The Cu distribution in different fractions on sludge amended soils is one of the factors affecting Cu mobility. To have an accurate description of Cu speciation in sludge amended soils, sequential extraction (BCR) were used to identify changes associated with Cu forms after batch test experiments. The results were presented in Figure 61.

Conventional activated sludge samples:

After the batch tests, as expected the copper distribution in exchangeable fraction (F1) is reduced to zero or to very low values. Moreover the results of BCR extraction method after sludge adding showed that Cu distribution is different when comparing with grass soil and paddy soil (Figure 61).

When sludge was amended on **grass soil**, almost Cu stayed in F4- residual fraction, followed by around 10% Cu linked with (F3) fraction, small amount of copper were distributed. Cu was in F2 and F1. This important Cu distribution in residual fraction (F4) in sludge amended grass soil can partly explain low Cu desorption percentage in grass soil.

A different copper distribution was obtained in case of **paddy soil**. In control soil, the copper bound F1 fraction does not exist and the Cu linked F2 was very low. In landspreaded soils, Cu forms did not appear in F1 fraction, except for BCh and the distribution of Cu in F2 was very low. In the case of BCh, the more important distribution of copper in F1, F2 fraction (compare to other sludge samples) can explain the high



percentage of Cu desorption when BCh was amended on paddy soil and proved that copper was continuously transferred to available fractions.

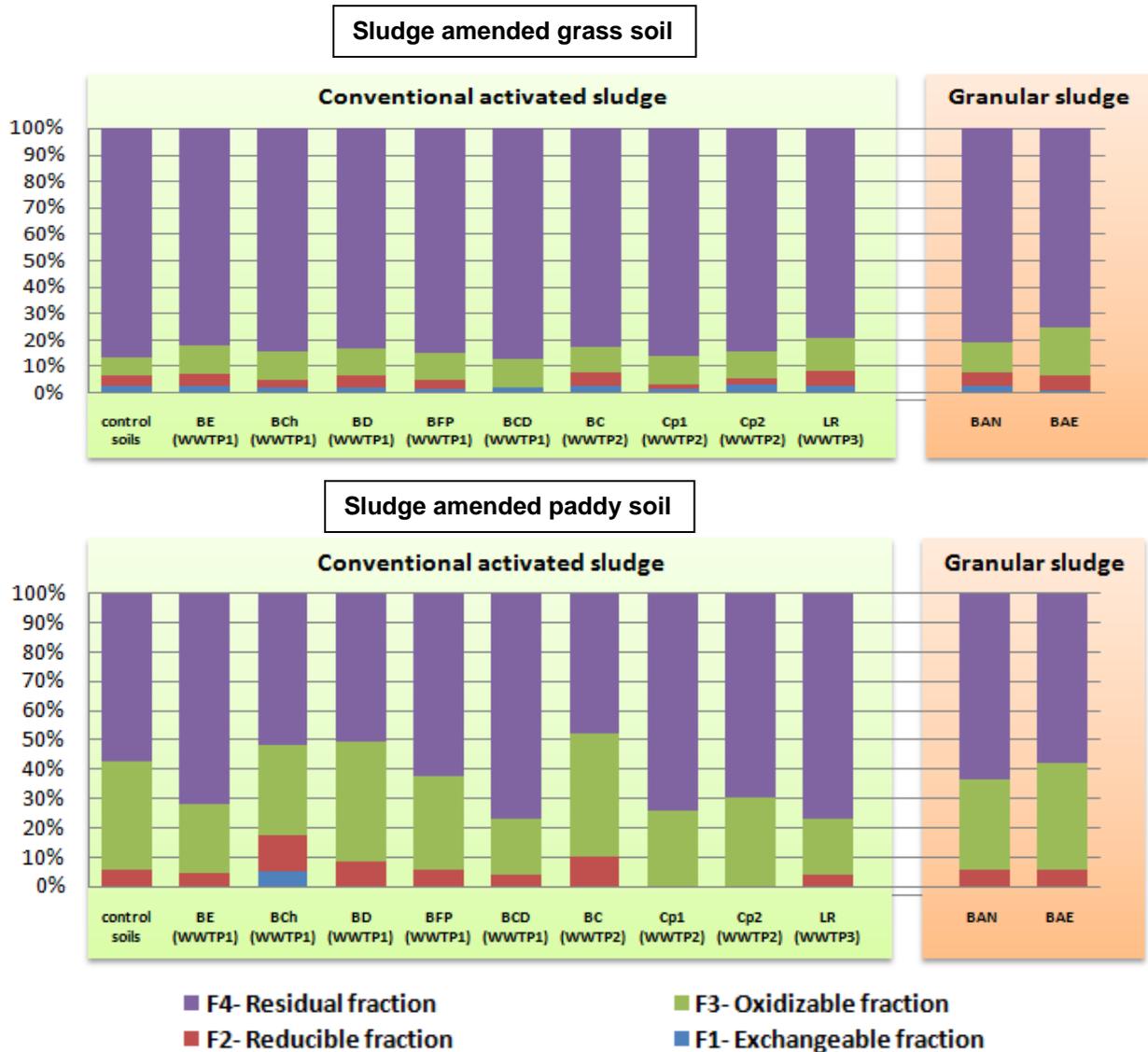


Figure 61 : Residual Copper distribution on sludge amended soil after desorption batch test in the different BCR fractions

In **paddy soil**, around 20-30 % of total Cu was distributed in F3 fraction. Cu binding with organic matter can be easily leachate in soluble phase, leading to an increase of the mobility of Cu, and to higher percentage of Cu desorption. In addition, in case of Cp1, Cp2, BE and LR, around 60 to 80% of copper is associated with the residual fraction which forms crystal part on the particulate sludge. This fraction enhances copper stability and implies that the Copper is not very mobile on sludge. It can explain why this sludge samples displayed low percentages of copper desorption. The similar effect also obtained when they were applied on soils.



Granular sludge:

The distribution of copper in the different fraction was also measured for anaerobic and aerobic granular sludge amended soils. In control samples of the two soils, the percentage order of Cu distribution in the different fractions was: $F4 > F3 > F2 > F1$. In granular sludge amended soils, the residual fraction always represented the largest residual fraction (F4) ranged from 56 % to 63 % for paddy soil and from 75 to 81% for grass soil for BAN and BAE amendment respectively. The reducible or Fe-Mn oxide fraction (F2) of Cu was the lowest in the BAN amended both soil compared to BAE.

In **grass soil**, copper distribution of BAN landspreaded soil was quite comparable to control soil, while organic matter fraction (F3) was sensibly more important in BAE and BAN landspreaded soil. The increase of copper in F3 fraction was detrimental to residual fraction (F4). This probably indicated that the mobility of Cu increase in grass soil when it was amended by granular sludge, probably due to dissolution of organic matter content during mineralization. This result can partly explain why Cu presented more leaching ability (in grass soil) in case of granular sludge amendment.

In **paddy soil**, copper distribution in the different fraction of granular sludge landspreaded soil is completely comparable to soil control. Nevertheless, results of Figure 60 showed that copper release was lower when granular sludge was amended to soil compare to control soil. Therefore, Cu is more stable in granular sludge amended paddy soil.

In case of **granular sludge** landspreaded soils, BCR fractionation cannot explain itself copper release efficiency.

II.7. Conclusion

In the previous chapter, 9 samples of stabilized sludge from conventional activated sludge processes and 2 samples from granular sludge processes were characterized. In this chapter sludge characterization was used to interpret interactions between these sludge samples and copper. Cu was chosen as this component is widespread in sludge all around the world, causing some problems in terms of important concentration, sanitary risk and is easily leachate when landspreaded.

As landspreading is the elimination route chosen in this study for sludge valorization, two kinds of soils were studied representing partnership between 2 countries: grass soil from Limousin region in France and paddy soil from Vietnam.

Sorption and desorption constant were assessed under batch conditions for sludge and soil alone and also for sludge and soil mixing. The measurements were implemented under batch conditions. The obtained results were analyzed with the objective of “predicting” Cu fate in case of landspreading. The final goals are being to propose some recommendations in terms of sludge stabilization process and wastewater treatment (CAS or GRANULAR) in the context of a developing country, like Vietnam.



Sludge samples sorption ability:

- For conventional sludge, the order of sorption ability was the following: composted and centri-dried sludge > reed bed > digested > thickened > centrifuged sludge. Liming and filter-pressed sludge processes led induced a strong increase of pH and positive cation, which were entering in competition with copper ion and thus reduced the copper adsorption ability.
- Anaerobic and aerobic granular sludge had similar and high tendency for copper adsorption capacity.

It was possible to demonstrate that sludge particulate and soluble chemical functionality, sludge organic matter content, CEC and biochemical composition were useful tools to explain copper sorption ability.

Soil sorption ability:

Grass soil exhibited higher copper sorption ability compare to paddy soil. These phenomena could be explained by the higher clay and organic matter content of grass soil.

Soil and sludge/soil mixing samples sorption ability:

The copper sorption ability were investigated for different sludge application rates (at 3, 30 and 150 tons DS/(ha.year) at L/S ratio of 10 (L/kg)) under batch test conditions

- Linear or Freundlich parameters could be good indicators to described copper adsorption behavior for all sludge application rates. However, they were not accurate for calculation of the maximum copper capacity according to Langmuir model. The fate of copper distribution in particular sludge matrix could be indicated by isotherm adsorption were linear.
- Sludge amended grass soil had higher Cu adsorption ability compare to sludge amended paddy soil at any sludge application rates.
- The only parameters which were really affected by sludge amending rate were q_{\max} and K_d and K_f for grass soil and only q_{\max} for paddy soil.

Conclusions about desorption ability:

The analysis of copper desorption made it possible to determine the mobility of copper in sludge and soil alone and in sludge amended soils. The sludge types and sludge application rate had significant impact on Copper mobility.

- High sludge application rate of 150 tons DS/(ha.year) resulted in high copper release due to high copper desorption efficiency.



- Copper was significantly released from limed, filter-pressed sludge alone and from sludge amended both grass and paddy soils. Other conventional sludge, especially composted sludge, showed lower copper desorption efficiency from sludge and sludge amended two soils.
- Granular sludge had high affinity for copper but they presented different trend for copper desorption. Higher copper release was measured in aerobic granular sludge compare to aerobic granular sludge not only for native sludge but also for sludge amended grass and paddy soils.

Conclusion about copper distribution after desorption in sludge amended soils: BCR extraction

The copper distribution in sludge amended paddy soil after batch leaching test was influenced by the kind of sludge samples and differed compare to sludge amended grass soil.

The copper distribution in sludge amended grass soil was quite comparable whatever the sludge sample and was comparable to control soil.

In grass soil from France copper desorption is increased by granular sludge landspreading compared to conventional sludge especially in the case of digested, limed, thickened and filter-press sludge

In case of paddy soil from Vietnam, it is particularly interesting to note that granular sludge landspreading allowed to reduce copper desorption compared to conventional sludge samples





III. Chapter 3: Fate of copper during landspreading of conventional and granular originated sludge samples: laboratory soil columns experiments.

Chapter 2 was devoted to the study of the Cu adsorption/desorption on different sludges, soils and sludge amended soils following the batch tests. Some tendencies were lightened in regard to interactions between copper soil and sludge and the importance of an accurate study was evidenced.

However, the information obtained is limited because some other conditions such as the hydrodynamic and weather condition effects cannot be taken into account in these experiments. Instead, column experiments are often performed in order to more closely reproduce field conditions and may provide information those are not available from batch experiments.

Due to the application of sludge in soil, heavy metal such as Cu is accumulated or leached depending on various factors related to human activities but also to the effect of environment such as weather conditions, soil characteristics and sludge amendment properties, etc. Normally, tropical weather condition (such as Vietnam) led to different metal mobility in soil comparing with temperate zones (such as France).

Then according to the results of chapter 2, if the heavy metals can leachate under lower rainfall condition for example of in Limousin region, this ability may be increased at higher rainfall condition such as South Central coast region in Vietnam due to different soil properties. In particular, the sandy soil nature can increase these phenomena (Elkhatib and Moharem, 2015). In this study, the paddy soil from Vietnam and grass soil from France were compared. Different sludge samples were landspreaded and the influence of stabilization process as well as sludge origin (CAS versus granular) was investigated. The conditions of sludge land-spreading were in accordance with the French regulation (3 tons DS ha⁻¹) as there is no regulation for the moment in Vietnam.

Sludge amending can affect some factors such as pH, redox potential (RP), electric conductivity (EC) and dissolved organic matter (DOM) which could explain the effect of sludge and soil characteristics on Cu mobility when sludge amended soil columns. These points will be discussed in this chapter.

The objectives of this chapter were double:

- Evaluating the potential leachability/accumulation of Cu in grass soil and paddy soil following sludge application (rate of 3 tons DS sludge ha⁻¹ year¹);
- Identify the distribution of copper in the different soils layers using the sequential extraction procedure.

Figure 62 presents the diagram of the experimental and scientific procedure driven in this chapter.



The experiments described above were carried out for 1) the nine CAS samples and the two granular sludge samples and for 2) the two soils: grass and paddy soils. In parallel a column without sludge landspreading was implemented to serve as control.

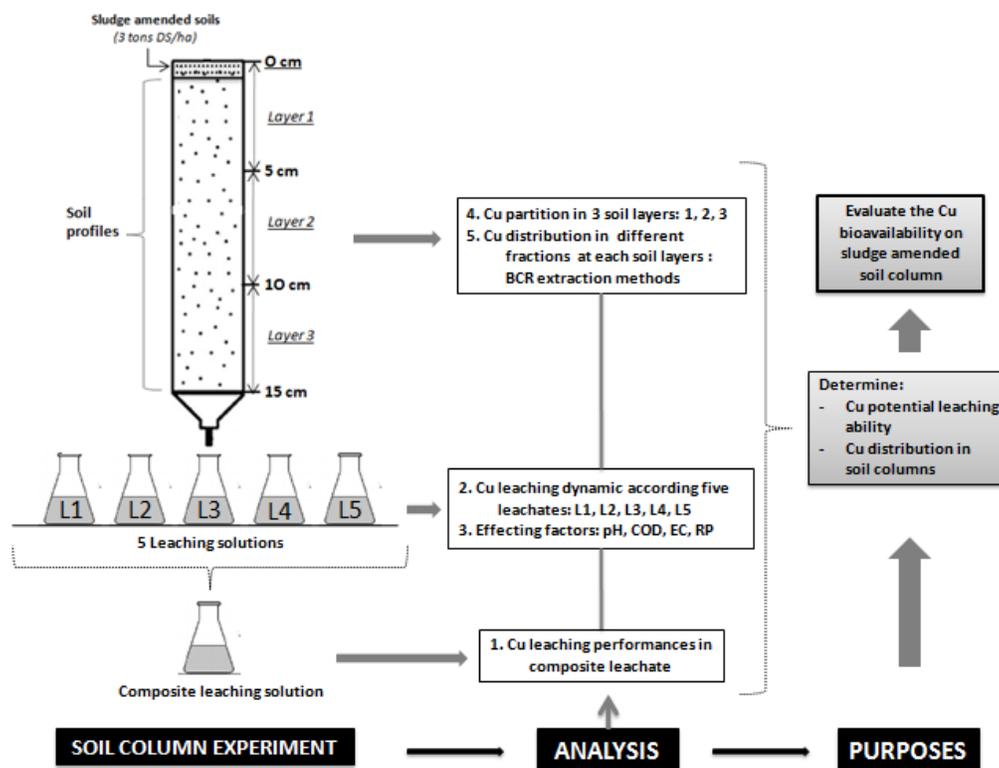


Figure 62 : Summary the objectives of chapter 3 for the study of the fate of Cu in sludge amended soil columns

III.1. Copper concentrations and leaching performances during lab-scale columns experiments

When sludge was applied on soil column, the copper leaching ability was determined by measuring copper concentration each two days. At the end of the experiment, a composite sample (the leachate was collected and mixed at the end of the leaching experiments) was done. The Cu concentrations in the composite leachate are reported Table 33. These values were compared to the amount of copper leached from the control soil columns. The Cu leaching performances on sludge amended soils were calculated based on the Equation 21 (See part 2-III.3, page 111).

III.1.1. Cu concentration in the composite leachates

The Cu concentrations in final composite leachates were presented in Table 33. Comparing results obtained for sludge amended soils and control soils, the application of sludge to grass soil and paddy soil caused an increase of total contents of Cu in the leaching solution.

For a given sludge sample, the Cu concentration in leachate depended on soil origin: the Cu concentrations in paddy soil leachates are always lower than that in grass soil leachates for both of control soils and sludge amended soil columns. The higher Cu concentration in initial grass soil (Table 27, chapter 2) could partly explain the reason for higher Cu concentration in grass soil leachate.

This experiment showed that, despite the differences, all the sludge samples can be landspread on grass soil or paddy soil in regard to groundwater pollution. Total Cu concentration in mixed leachates was below the maximum amount of Cu authorized limits for drinking water standards according to the European legislation – 98/83/EC (2 mg/L) and Vietnamese legislation for surface water standard - TCVN 5942-1995 (1 mg/L) (Appendices 4).

Table 33: Cu concentration in total leachates after leaching process

Sludge amended soils		Cu concentration (mg/L)	
		Grass soil	Paddy soil
Control soils		0.029	0.007
Conventional activated sludge	BE (WWTP1)	0.150	0.013
	BCh (WWTP1)	0.152	0.040
	BD (WWTP1)	0.130	0.030
	BFP (WWTP1)	0.138	0.027
	BCD (WWTP1)	0.053	0.016
	BC (WWTP2)	0.049	0.029
	Cp1 (WWTP2)	0.052	0.009
	Cp2 (WWTP2)	0.048	0.010
	LR (WWTP3)	0.116	0.008
Granular sludge	BAN	0.198	0.016
	BAE	0.230	0.015

III.1.2. Cu leaching performances

The percentage of Cu desorption could be a good indicator to predict Cu potential for leaching. Percentages of Cu desorption of sludge amended soil profile depended on the kind of sludge applied (Yang et al., 2017) and on the properties of soil (Wong and Selvam, 2006).



Grass soil and paddy soil leaching behavior were compared to non-amended soils (control soils) (Figure 63). The copper leaching percentage was calculated according to (Equation 21) (See part 2, page 114). The percentage copper leached from the soil column was calculated based on the amount of copper released in comprise leachate of five leaching solutions at the end of leaching process per mass of copper in soil column at starting time. Whatever samples, very low leaching percentages were measured. Nevertheless, the results evidenced effect of the sludge amending and effect of the soil origin on leaching performances.

These results are discussed thereafter.

Figure 63 - The Cu leaching percentage for granular and conventional sludge amended on grass soil and paddy soil.

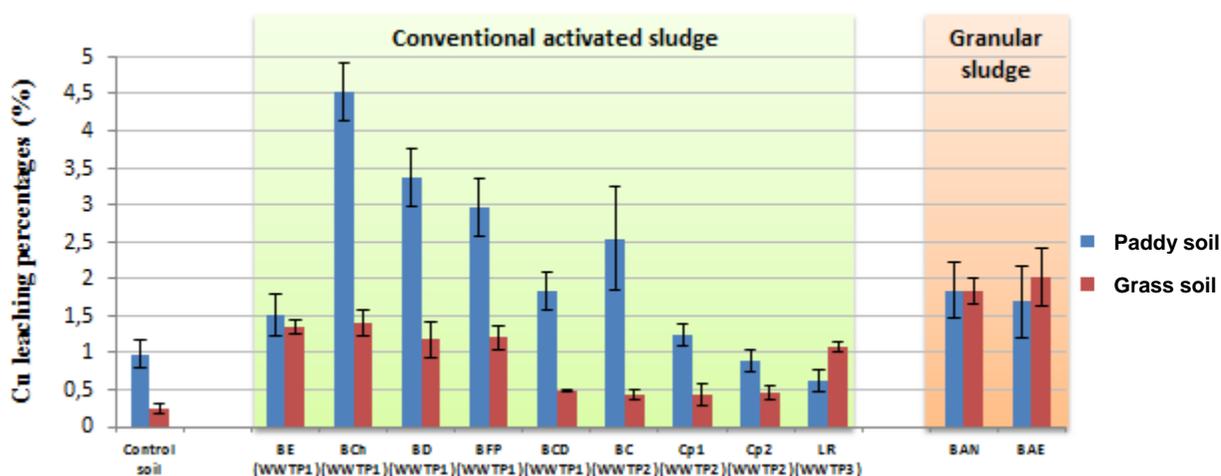


Figure 63: Cu desorption efficiency on granular and conventional sludge amended grass soil and paddy soil columns.

III.1.2.1 Impact of soil origin

The result reported in Figure 63 highlights that paddy soil had higher Cu release ability for most of the samples (including control) compared to control grass soil. Nevertheless, in some cases, paddy soil release ability was comparable to grass soil and especially in the case of granular sludge. This result constitutes a positive point for using granular sludge processes in Vietnam for further sludge valorization.

It was demonstrated in Table 27 (Chapter 2, page 144) that Cu speciation was comparable for control paddy soil and grass soil, However, the results in terms of leachability are at least different, for a given sludge sample. Some hypothesis on these differences can be drawn such as soil composition.

This phenomenon can be explained by higher clay contents in grass soil compared to paddy soil. Zheng et al., (2012) showed that adsorption on clay and organic matter with very high-binding energies impacted to decrease the heavy metal movement through the soil columns and thus decrease the leaching



performances. Moreover, Toribio and Romany (2006c) demonstrated that soils with high amount of clay had the highest metal retention capacity while loam soils with low clay content had the lowest. Besides, the organic matter on soil also effect to the Cu leaching ability. Mattana et al., (2014) showed that leaching ability of heavy metals (Cu, Ni, Pb, Cd and Zn) was higher in the sand soils compare to the sandy loam soil.

These results are consistent with the hypothesis that the lower organic matter and clay content of the sandy soil limits metals binding capacity.

Copper leaching in grass soil was always lower or at least equal to copper release in paddy soil. This result showed that grass soil composition acted as a retention system for copper. This result is easily explained by higher CEC in grass soil. The grass soil has a high ability for cationic exchange compare to paddy soil (Table 26).

Interestingly copper release is comparable for the two soils amended with granular sludge.

In addition, soil pH also affects to desorption of heavy metals in soils. The results of (Kandpal et al., 2005) suggested that the acidic soil pH might reduce the ability of the soil to naturally sequester heavy metal cations and lead to increase their leaching. In this study the soil pH was quite comparable and rather acid so this parameter cannot directly explained the discrepancies.

In this study the higher Cu leaching capacity in paddy soil can be explained by low pH value, low clay and organic contents and low CEC.

III.1.2.2 Impact of sludge origin on copper leaching

Conventional sludge amended soils

- **Sludge amended on grass soil**

The order of Cu leaching percentages is: BCh > BE > BFP > BD > LR > (BCD, Cp2, Cp1 and BC). Cu desorption percentages varied from 1.39% for BCh followed by leachate from BFP, BE, BD and LR with 1.21%, 1.35%, 1.18% and 1.07%, of leaching percentage respectively. In the case of BCD, BC, Cp2 and Cp1 sludge amended grass soil columns, the leaching percentage was negligible (around 0.4% – 0.5%).

- **Sludge amended on paddy soil**

The Cu leaching percentages followed the order: BCh > BD > BFP > BC > BCD > BE > Cp1 > Cp2 > LR.

As for grass soil, BCh amendment presented the highest Cu leaching efficiency with 4.51%. The leaching values varied from 4.51% to 0.6%. Cu release percentages were respectively equal to BD (3.36%), BFP (2.97%) and BC (2.55%). There are small differences of Cu desorption efficiency in leachates of Cp1, BCD and BE



sludge amended soil columns when compared to control soils: the values varied around 1.24% (Cp1) to 1.83% (BCD). With 0.61% and 0.89% of Cu leached percentages, LR and Cp2 amended paddy soil, exhibited the lowest (negligible) Cu mobility.

In the literature, the ability of soil from Vietnam to release Cu after adding fertilizer was already demonstrated. For example, Marcussen et al., (2008) investigated Cu leaching (CaCl₂ 0.01M) from Vietnamese soil collected from agricultural land (sampled in peri-urban Hanoi city) irrigated by peri-urban wastewater. They found more than 34% of Cu leaching. In other study of Buekers et al., (2007) confirmed the small amounts of Cu fixed in the soil, due to high Cu leaching under low pH values of acid soil after fertilization.

Whatever the soil origin composted (Cp2 and Cp1), centri-dried (BCD), centrifuged (BC), reed-bed (LR) sludge presented low Cu release (lower than 2%). The low leaching ability of composted sludge and dried sludge in regard to copper has been widely reported in the literatures (Fang et al., 2016b; Mattana et al., 2014b). In contrast, filter-pressed (BFP), digested (BD), thickened (BE) and limed (BCh) sludge amended soils increased Cu availability in soil. Qi et al., (2011) also showed that press-filter sludge amended soil can increase the Cu leaching ability. Leachate concentrations varied from 84 µg/L (in leachates of control soil) to 100 – 140 µg/L in the leachates of press filter sludge amended soil.

Granular sludge amended soils

Cu leaching was more important when soil was amended with granular sludge compare to the non-amended soils. In case of grass soil, both of anaerobic and aerobic granular sludge amendment increased significantly the copper desorption percentages, which were equal to $2.03 \pm 0.13\%$ and $5.75 \pm 0.32\%$ respectively, compared to $0.86 \pm 0.18\%$ for non-amended grass soil. BAN and BAE presented very similar value of Cu leaching percentages on both of grass soil and paddy soil.

The copper leaching on granular sludge amended grass soil was often higher than those measured for conventional sludge samples. In the case of sludge BAN and BAE amended paddy soil, leaching percentage was slightly higher than some conventional sludge samples amendment such as composted (Cp1 and Cp2), thickened (BE), reed bed (LR) and centri-dried sludge (BCD), but lower than limed (BCh), filter-press (BFP), digested (BD) and centrifuged (BC) sludge on Cu leaching percentages. Thus granular sludge landspreading limit desorption in paddy soil.

III.1.2.3 Impact of leaching tests implementation (Batch/Columns)

The interest of batch tests in terms of prediction of Cu leaching ability was investigated. Therefore columns results were compared to batch results. In theory, batch tests provide a snapshot of particular experimental conditions of L/S ratios, agitation rates. Column tests, at other sides, enable time-dependent monitoring of Cu leaching from soil columns; in addition, flow-through pattern of such test mimics environmental conditions. On Figure 64, the Cu leaching percentages obtained either in batch either in



columns conditions was compared for the two different soils. In the control soils, paddy soil showed higher leaching ability compare to grass soil whatever the kind of implementation.

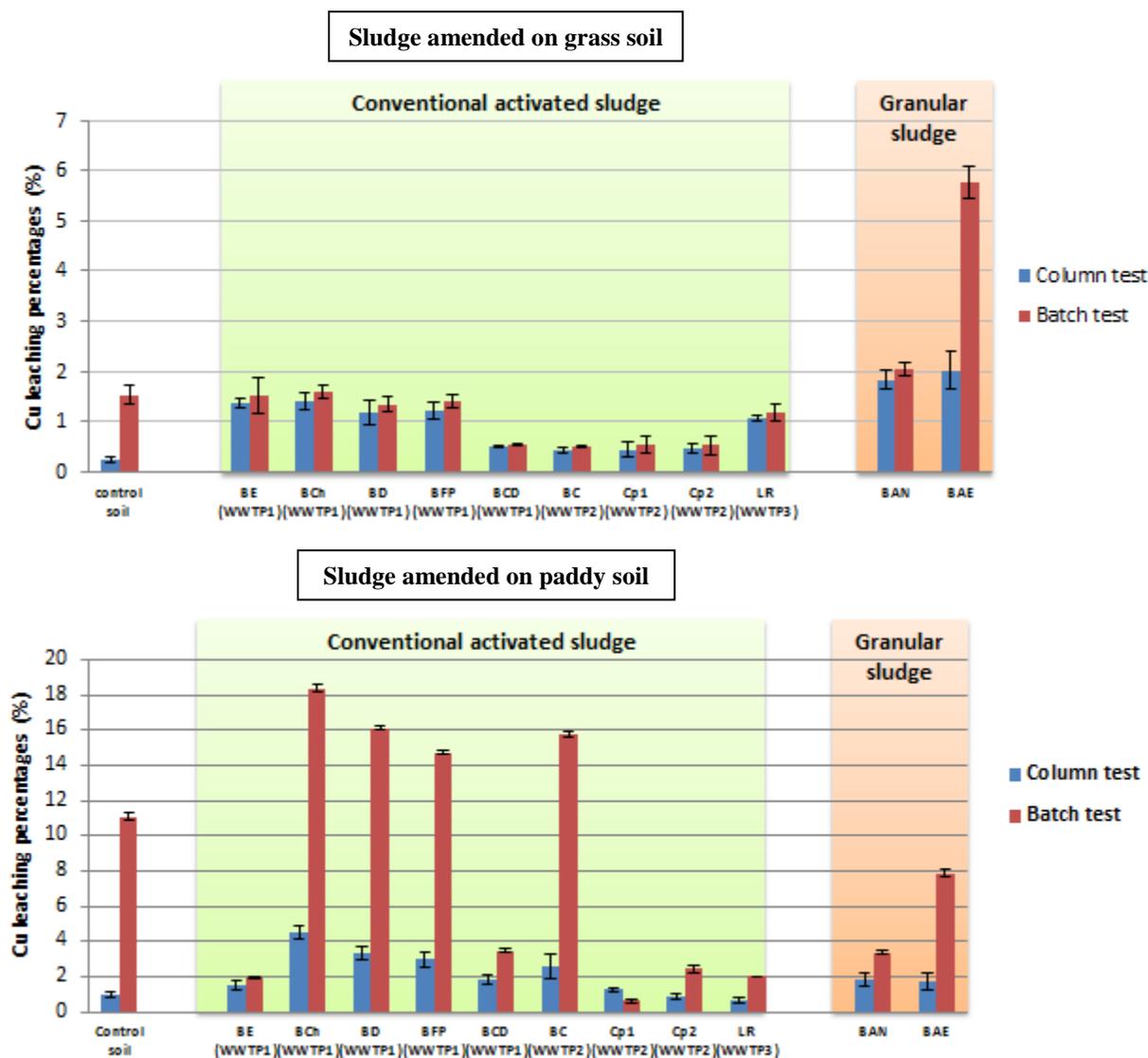


Figure 64 : Comparison of Cu leaching percentages on sludge amended soils: either under batch or columns conditions

Conventional sludge:

In the amended grass soils, the order of copper leaching in columns was very closed from the results obtained from in batch conditions (Chapter 2) which was : BCh > BD ≈ BE > BFP > LR > Cp1 ≈ Cp2 ≈ BC ≈ BCD. But for some sludge samples (BAE, LR, BFP), batch measurements led to an over estimation of copper leaching.

In the sludge amended paddy soil, the order of copper leaching in batch conditions were also much closed to those obtained in columns. In batch tests (chapter 2), the order of copper desorption percentages



was: BCh > BFP > BD \approx BC > BCD > Cp2 \approx LR > BE > Cp1. But for most of the sludge samples (excepted BCD, Cp1 and Cp2), batch measurements led to an over estimation of copper leaching ability.

Batch conditions can give be used for a rapid prediction about copper leaching ability tendency but cannot be used for direct leaching percentage measurement. This is possibly due to the difference in the experiment procedure (over end tumbling for batch tests as opposed to preferential up-flow for the column tests).

Granular sludge:

For conventional activated sludge, that batch tests could be interesting to draw a tendency of copper release but the real leaching percentage has to be determined under dynamic conditions of soil column (López Meza et al., 2010).

In the case of granular sludge, batch tests led to an over estimation of copper leaching ability, especially in the case of BAE and cannot even be used to draw a tendency.

III.2. Investigations about copper leaching behavior according to the leaching time: dynamic approach

The copper leaching behavior following sludge landspreading is essential to the assessment of the associated risk of soils, and hence to the optimization of sludge application for agricultural activities. In this study, leaching volume of flushed solution was collected every two days during the ten days of the experiment. The objective was to reproduce a one year precipitation in Limousin (France).

The copper concentration in each leachate was determined and thus the Cu leaching behavior was evaluated along time. Figure 65 represented the copper concentration in five leachate solutions: L1- 1st; L2 – 2nd; L3 – 3rd; L4 – 4th and L5 – 5th solutions, which respectively corresponded to solutions collected after 2 days, 4 days, 6 days, 8 days and 10 days. The measurement was done in duplicate. For one soil column, the Cu leaching concentration in leachate will reflect the Cu leaching dynamic through soil column.



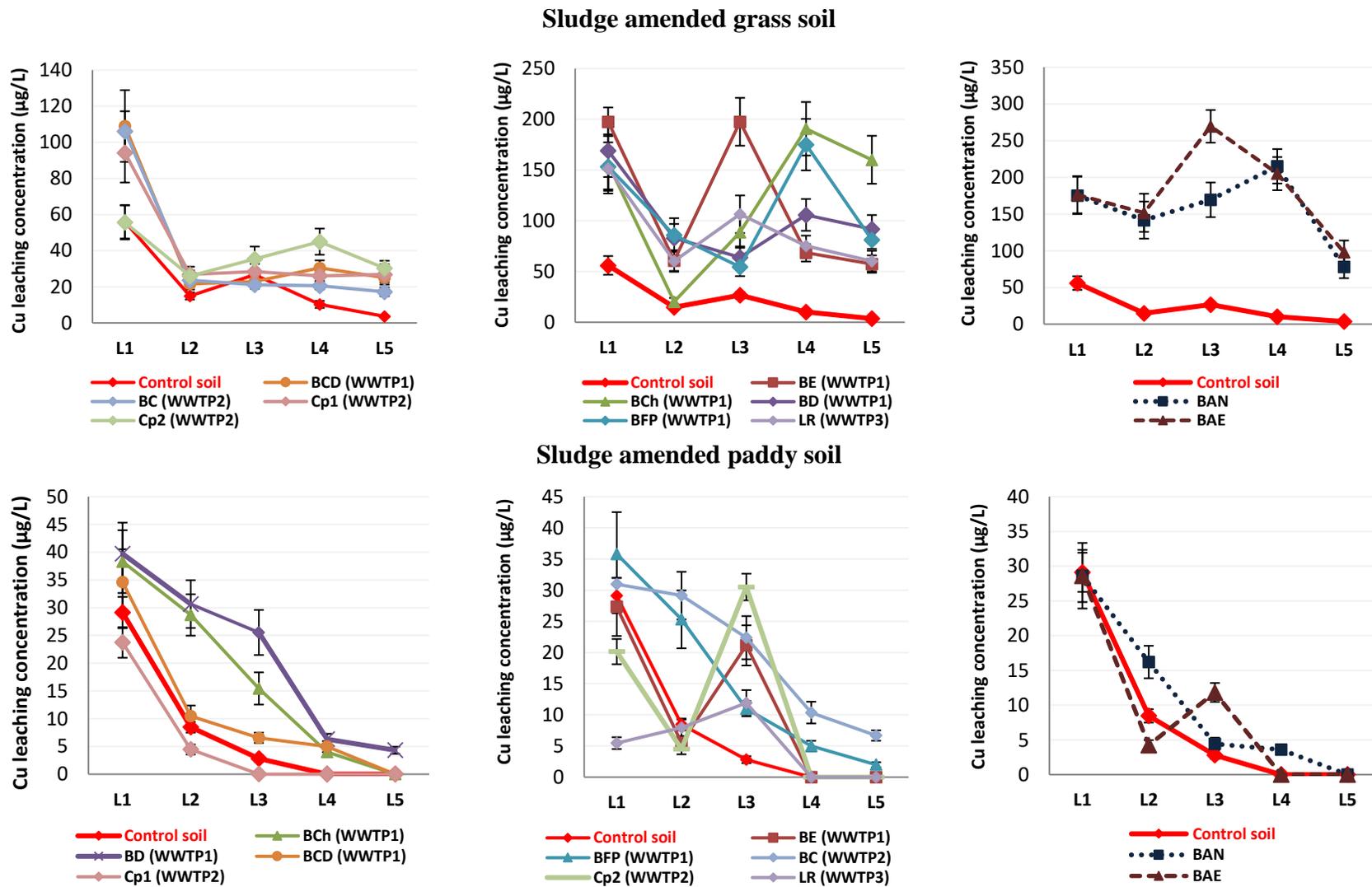


Figure 65 : Cu concentration in five leachates collected a long time from conventional activated/granular sludge amended grass soil and paddy soil



- **Non-amended soil columns**

Leaching solution induced Cu moving downward rapidly during the first two days then slower during next days for both grass soil and paddy soil. The most important leaching efficiency occurred in the first leaching (L1) event for almost samples. In the first leachates (L1), the Cu leaching concentrations were 29.12 µg/L and 56.01 µg/L for grass soil and paddy soil, respectively. These values corresponded to 50% and 80% of Cu leaching percentages (Appendices 3), for grass soil and paddy soil column, respectively. The Cu leaching behavior in the control soils consisted in two phases: an initial rapid leaching followed by a slow decrease. In the paddy soil, there was no more leaching after 3 days.

- **Sludge amended columns**

The Cu leachability evolution with time is highly dependent on the kind of sludge applied and of course the kind of soil. This phenomena evidenced that Cu behavior in sludge amended soils is affected by a number of complex processes including sorption onto surface of soils, complexation with inorganic and organic ligand, precipitation and so on (Xu et al., 2005). These interactions are discussed later using the BCR protocol.

The copper leaching dynamics was also investigated in the objective of comparing 1) the impact of sludge process stabilization treatment (case of CAS) and 2) the dynamic of copper in case of granular sludge landspreading.

III.2.1. Copper dynamic in soils columns amended with stabilized Conventional Activated sludge (CAS)

Case of sludge amended grass soil

Except in the sample Cp2, the leachates collected from CAS amended grass soil had higher Cu concentration compare to the non-amended soil column.

The evolution of Cu concentration in leachate along time for samples Cp1, Cp2, BC and BCD presented comparative trend compare to control soil (Figure 65): Cu leaching was high in first days (until day 4th), then Cu concentration in leachate stayed stable until the end of the experiment. More than 50% of Cu was released in the first leachate and 15-20% in the next of leaching process (See Table 37-Appendices 3).

In soil column amended by BCh, BD, BFP, BE, LR, a different behavior was observed (Figure 65). Copper concentration rapidly decreased during the first 4 days, then its evolution was quite different for each sample (either increase or decrease) and it is difficult to find a similar trend. At the end of the experiment copper concentrations were largely higher to the control. It can be hypothesis that there is a slight retardation of Cu leaching due to copper interactions with sludge.



Case of sludge amended paddy soil columns

Paddy soil columns experiments revealed very different behavior along time according to the sludge sample landspreaded so that it is not possible to draw a general tendency.

In case of Cp1, BD and BCD, Cu leaching concentration evolution along time showed quite comparable trend to the control. Copper was highly released in the first days (L1 and L2). Then Cu was not detected or under detection limits in last leachates. It evidenced that copper moved fast in paddy soil columns during leaching experiments.

The case of composted sludge as to be considered separately as copper leaching was very low compare to other kind of stabilized sludge samples. The low leaching of copper in composted sludge has already been reported and is related to the specific adsorption of Copper on composted organic matter (Torri and Lavado, 2008 ; Kunito et al., 2001a; Rodríguez-Vila et al., 2015).

In the case of all other sludge amended paddy soil such as BC, BE, BFP and LR, the Cu leaching concentration in leachates collected after 4 days (L2) and 6 days (L3) was high and corresponded to around 40-60 % of Cu leaching ability (Table 29). Then copper concentration decreased to less than 2.60 µg/L in leachates L4 in BE, LR and Cp2 amended paddy soil.

III.2.2. Copper dynamic in soils columns amended with Granular sludge

Case of grass soil:

Copper leaching trend was comparable for BAE and BAN, and differed from other samples. Cu leaching concentration was relatively uniform (around 170 µg/L) in three leaching solutions (L1, L2 and L4) and decrease to approximate 90 µg/L in solution L5. The Cu leaching concentration showed the highest values 169.47 µg/L and 215.12 µg/L in leachate L3 and L4 when BAE and BAN amended grass soil, respectively. This behavior deferred completely from the control but could be comparable to copper leaching behavior in columns amended with Cp2, BD, BFP and LR. It can be concluded that the addition of granular sludge modified Cu bioavailability in surface of grass soil and affects the Cu transport through the column (T. Pardo et al., 2014b).

Case of paddy soil

In BAN and BAE amended soil columns, copper leaching showed an evolution which was comparable to the control column: a gradual reduction in Cu leaching with time, which led to a disappearing of Cu in final leachates. Moreover, these trends observed on granular sludge amended paddy soil column was similar compared with those of conventional sludge amended soils such as BD, BCD, BFP and BCh.



These results demonstrate that the copper leaching dynamic was completely different according to sludge origin, stabilization treatment and according to the kind of soil. The movement of Cu in soils and sludge amended soils is not only greatly affected by the physicochemical forms in solid phases (Li and Shuman, 1997) but also influenced by many factors such as pH, dissolved organic carbon, electron capacity, etc., of leaching solutions which transferred through soil columns. Factor affecting copper mobility are investigated in next parts of this study.

III.3. Factors affecting copper mobility

As mentioned above, column experiments were designed to study copper leaching from sewage sludge amendment on soils. Theoretically, Cu leaching depends on its availability and mobility through the soil profile and sewage sludge application. In turn, the availability and mobility of Cu depend on mainly:

- Chemical properties such as soil pH and redox potential, which affect chemical speciation and solubility (Egiarte et al., 2006b);
- Solute-solute interactions such as metal complexation with either organic or inorganic species (Egiarte et al., 2006b);
- Soil surface characteristics and metal – soil interactions, which affects sorption reactions;
- Soil physical properties that affect water movement;

Metal complexation reactions and metal soil interactions are, in turn, affected by dissolved organic matter, further affecting metal solubility (Egiarte et al., 2006).

Hence, pH, electron conductivity (EC), redox potential (RP - See Appendices 3) and dissolved organic carbon (DOC) were monitored during leaching processes as potential indicators of copper leaching dynamic from soil columns (Figure 66).



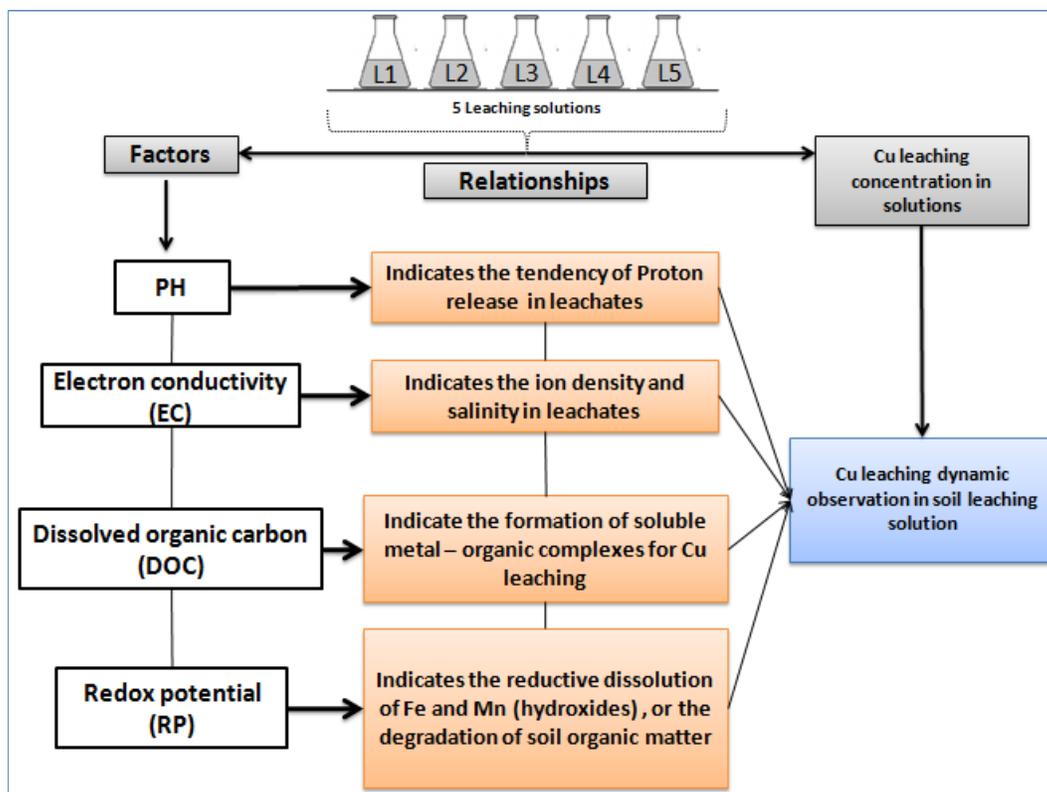


Figure 66 : Factors affecting copper mobility in soil and mechanisms associated.

III.3.1. pH variation during the leaching process

Cu is mobile both under acidic and highly alkaline conditions, but its mobility is usually the lowest at neutral to slightly alkaline pH (Fan et al., 2011). The mobility and toxicity of Cu could be reduced by raising soil pH and chemisorbing or precipitating Cu in soil (Fan et al., 2011). In soil with neutral pH, copper ion mostly bound to soil carbonates or remained in the residual fraction and their distribution did not significantly change between different soil layers (Suhadolc et al., 2010b).

Leaching process normally reflects affinity of contaminants with material incubated in the column. In soil, Cu is often associated with carbonates, phosphates, organic matter, oxides, clays and other silicate minerals (Fan et al., 2011). Its stability in soil is strongly pH dependent. Figure 67 showed the variation of pH of leachate which were collected every 2 days during leaching process.

- **pH of control soils leachates**

In this study, both grass soil and paddy soil are acid soils. The initial pHs are 5.6 and 5.2 for grass soil and paddy soil respectively (See chapter 2 - Table 27). The pH values of leachate L1 (collected after first 2 days) around 5.2 and 4.3, were lower to initial grass soil and paddy soil pH values. Moreover, the pH values in paddy soil leachates were lower than pH values in grass soil leachates.



The pH of leachates increased continuously with leaching time until reaching a plateau: between 6 and 6.5 for grass soil and between 4.5 and 5 for paddy soil. Despite close pH values for original grass and paddy soils, the pH of the leachate obtained under stationary conditions were very acid in case of paddy soil compared to grass soil. At the end of the leaching experiments, the pH values of leachate in paddy soil were closed to soil initial pH (from 5 to 5.5), while the pH values of leachate in grass soil were higher (from 6.5 to 8) than the pH of the control soil (pH = 5.6).

- **Conventional activated and granular sludge amended grass soil**

pH values were measured in landspreaded soil leachate in parallel of control soil leachate (Figure 67). These representations allow evaluating the effect of amending on pH leaching. In the case of grass soil, 3 different kinds of results can be described:

Figure 67a): Cp1, Cp2, LR and BE amending sludge samples have comparable pH evolution to that of the control soil, a plateau was reach at the end of the experiment. pH values in the leachate at the equilibrium showed that amending lead to a slight increase of pH to neutrality.

Figure 67b): BD, BFP, BC, BCD, BCh amending sludge samples had different behavior compared to the control, a plateau was not reached at the end of the experiment. Sludge amending led to an increase of pH during the length of the experiment. In leachate L5 they all tend toward a same basic pH value around 8.

Figure 67c): The specific case of granular sludge amending. BAE amending did not impacted pH evolution and value compare to the non-amended soil. In the case of BAN, pH also reach a plateau at the end of the experiment but leachate pH value was basic (pH around 8).

- **Conventional activated and granular sludge amended paddy soil**

As for grass soils, pH evolution of sludge amended paddy soil exhibited 3 different kinds of behavior in comparisons to the control (non-amended soil).

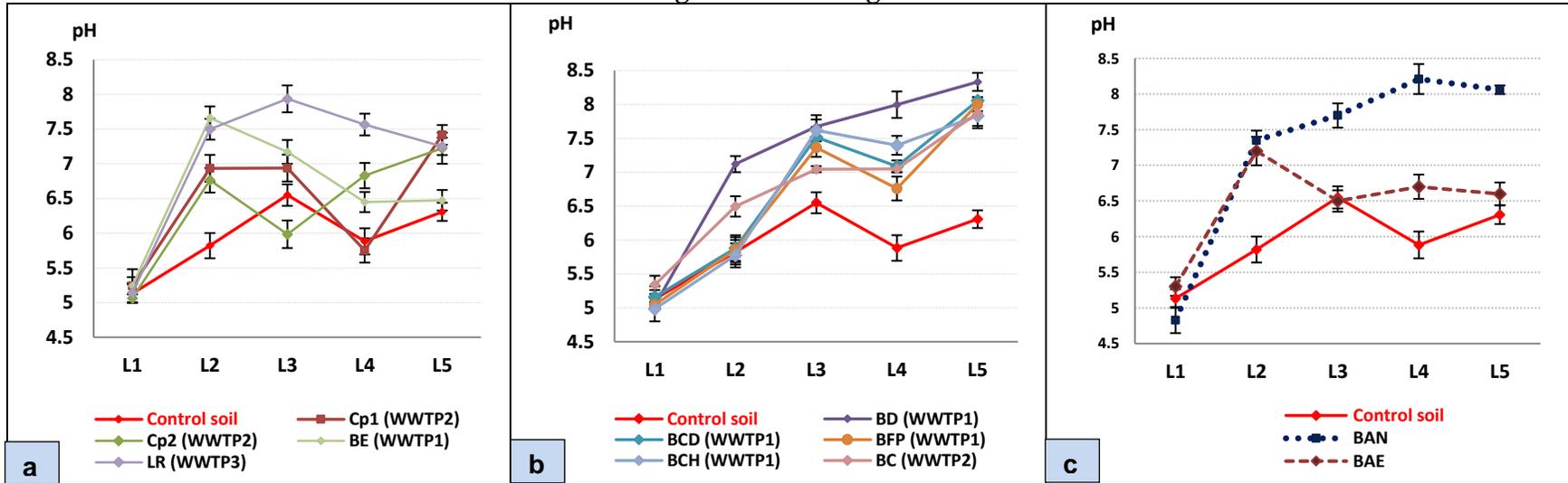
Figure 67d): BD, BFP, Cp1, BCD and BCh amending sludge samples have comparable pH evolution to that of the control soil, a plateau was reach at the end of the experiment. pH values in the leachate at the equilibrium were slightly more acid (pH lower or equal to 5) compare to control soil.

Figure 67e): BC, LR, Cp2 and BE amending sludge samples had comparable pH evolution to that of the control soil, a plateau was reach at the end of the experiment. pH values in the leachate at the equilibrium were slightly higher compare to control soil, but stayed acids (pH lower to 5.5).

Figure 67f): The specific case of granular sludge amending: pH evolution was comparable to control soil and a plateau was also reached, but granular sludge amending increased pH leachate values since the beginning of the leaching process. At the end of the experiment, the pH of the leachate was acid and lower to 6.



Sludge amended on grass soil



Sludge amended on paddy soil

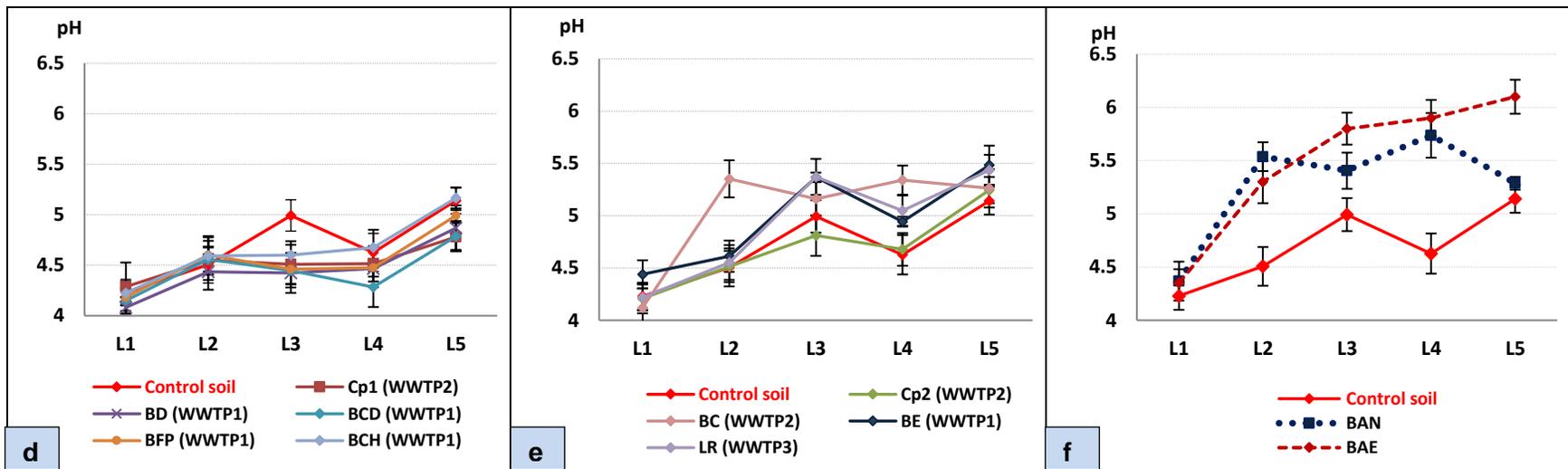


Figure 67 : pH evolution in sludge amended soil leachates and control soil. Case of paddy soil and grass soil



- Correlation between global pH variation and global Cu leaching efficiency

In order to assess to such extend pH variations affected total copper leaching, Figure 68 were drawn.

Grass soil leaching efficiencies covered a lower range compare to paddy soil. Grass soil, pH increase was more or less linked to an increase of copper leaching. But in the case of paddy soil, it was not possible to establish any correlation: pH values stayed constant (around five) whatever the copper leaching percentage.

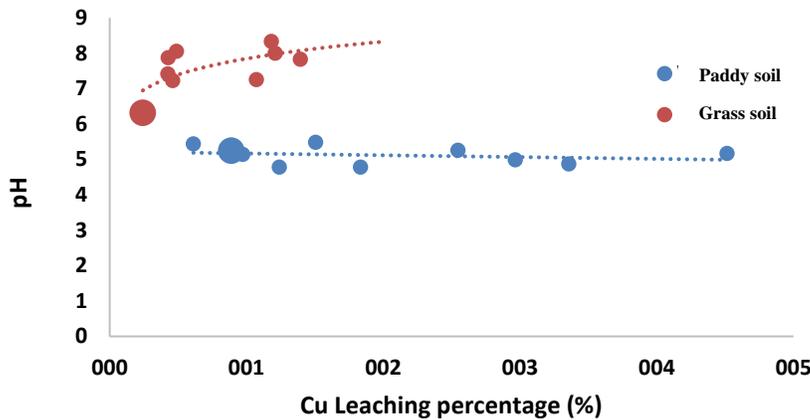


Figure 68 : Evolution of Cu leaching global percentage as a function of pH in leachate of the amended sludge columns (only conventional sludge). The biggest point represented the control soil.

Time /pH variations and copper desorption efficiencies were compared for a same sludge sample (BC) landspreaded either on grass soil either on paddy soil (Figure 69). In grass soil, Cu desorption concentration was clearly linked to increasing pH values, while in paddy soil, copper desorption led to slight pH variations. This result is in accordance with the general trend observed before on Figure 69.

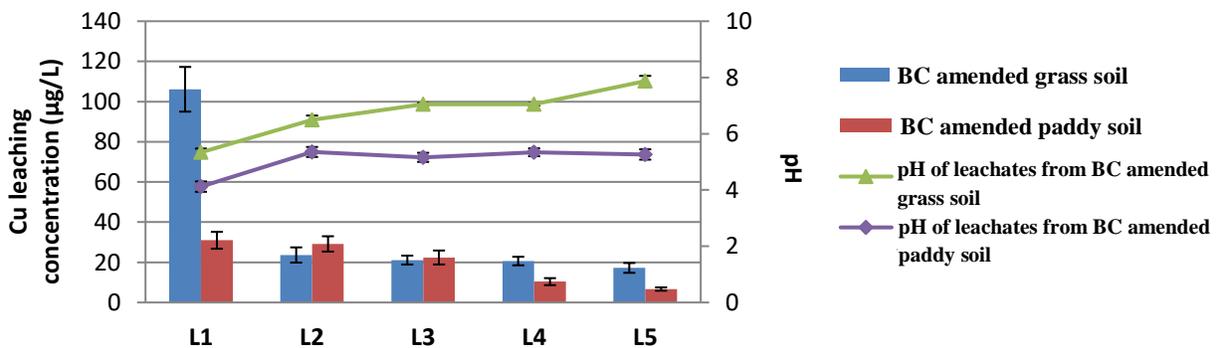


Figure 69 : Evolution of copper concentration (mg/L) and pH during column leaching tests: case of centrifuged sludge (BC) amended soil (grass soil and paddy soil)



- Discussion about the impact of pH on Cu leaching dynamic.

According to the literature reviews, pH values increase in soil solution could be due to anion fixation on iron and aluminum hydroxides or positively charged colloids linked to clay components of soil, this exchange lead to OH^- ion and negative group's release (Fontes and Alleoni, 2006). Moreover, the soil column were eluted by CaCl_2 0.01M, ion Cl^- can exchange with OH^- ion and negative groups on soils surface, lead to increase the pH values (Karak et al., 2005). At the same time, the Cu^{2+} ions could be precipitated with released OH^- or complexed with negative groups of soil ligands (Seshadri et al., 2015), which increase the Cu adsorption on soil surface and reduce the Cu leaching ability, which could explain the decrease of copper leaching efficiency along time.

In this study, for paddy soil, pH was not modified by the leaching process. However, for grass soil, all the final leachate pH values were higher than the leachate collected in the control. This phenomenon could be explained by higher CEC and clay content in grass soil, compared to paddy soil. During leaching process, the anion exchange mechanism may occur between Cl^- ion or negative groups of sludge and negative groups on clay component which increase the OH^- ion and negative groups release and increase pH but decrease Cu leaching efficiency.

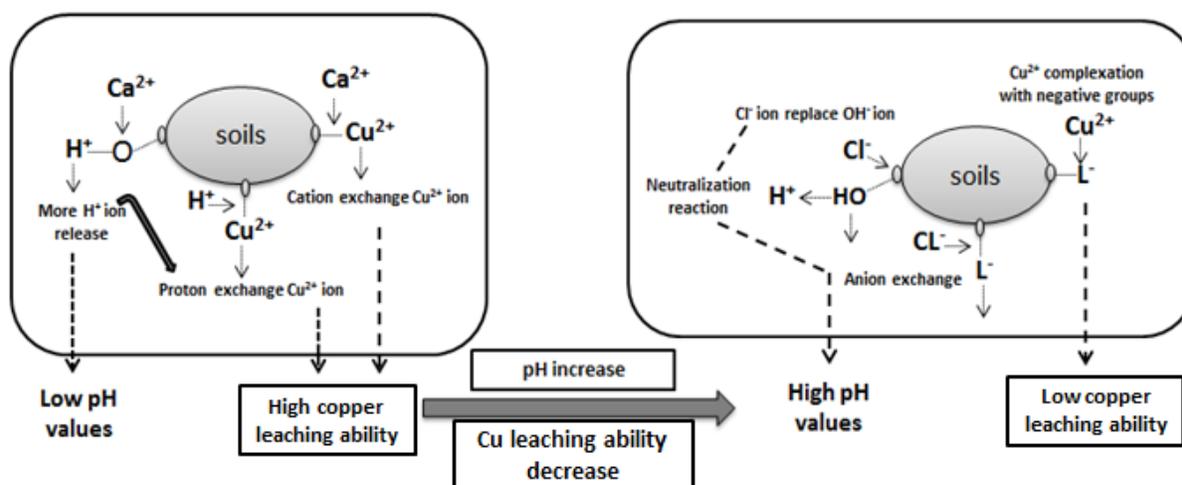


Figure 70 : Mechanism of pH on Cu release ability in grass soil.

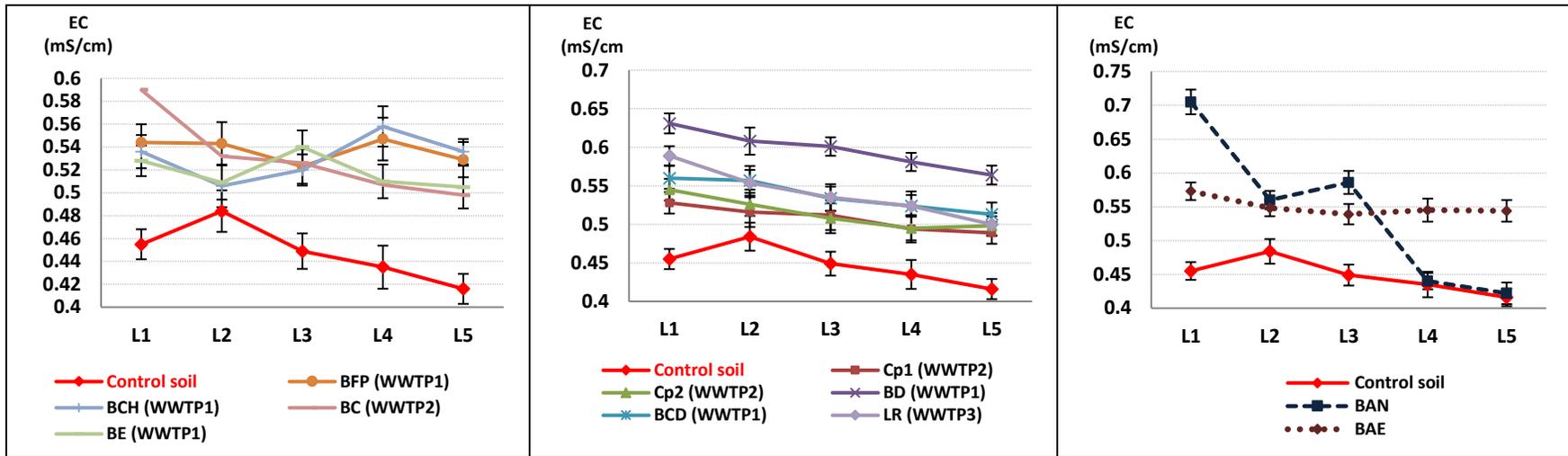
pH may explain Cu mobility, especially in grass soil, but not in the paddy soil. pH is not the only factor of influence. Others parameters like electric conductivity, dissolved organic matter were also measured.

III.3.2. Change of electric conductivity (EC) in leachates

The electric conductivity reflects the quantity of conducting particles in solution, which normally indicates the content of salts. The monitoring of this parameter is helpful to know the change of ion density in the leachates during the leaching process. Figure 71 shows the change of EC in the leachates during the course of the experiment



Sludge amended grass soil



Sludge amended paddy soil

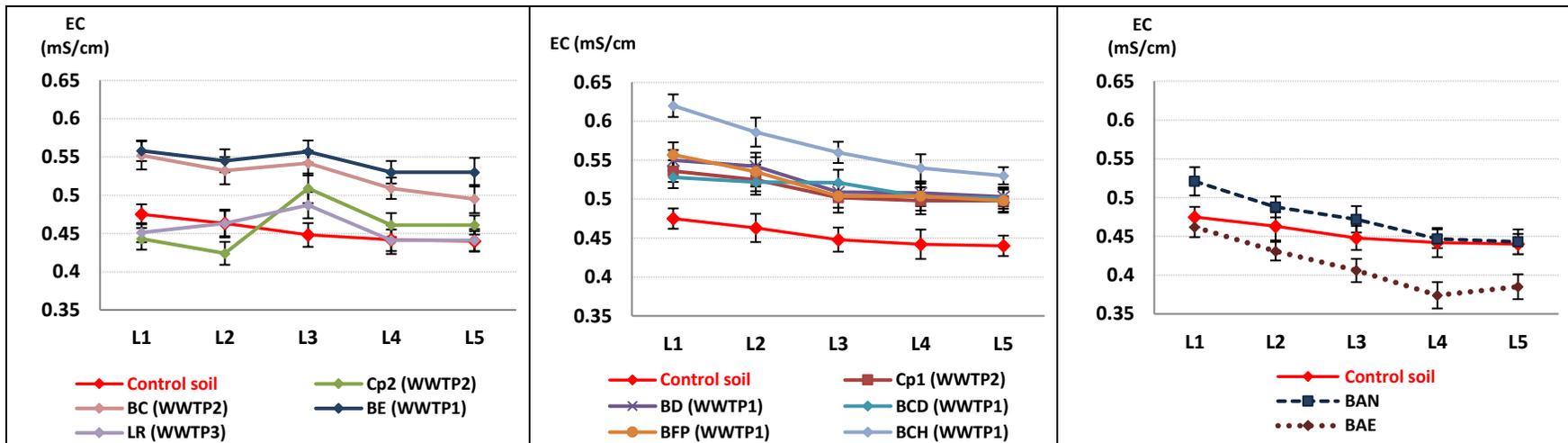


Figure 71 : EC evolution in sludge amended soil leachates and control soil. Case of paddy soil and grass soil



- **EC values of control soil leachates**

General tendency, the EC values of control grass soil and paddy soil decrease gradually from first leachates to fifth leachates. The EC values of non-amended soils stayed in the range of 0.4 – 0.5 mS/cm. There were no significant difference between the EC values in leachates of paddy soil and grass soil.

- **EC values of sludge amended soil leachates**

CAS amended soils

The effect of sludge stabilization process and amending on EC values were represented in (Figure 71). All sludge amended soils presented higher EC values in leachates compare to control soils. In almost case of CAS amended soils, EC of leachates continuously decreased during the leaching process. The decrease of EC indicated that the salinity in leachate decreased.

In the first 2 days period of leaching, the EC were obviously high in amended soils. They ranged from 0.45 to 0.6 mS/cm and 0.45 to 0.63 mS/cm in cases of grass soil and paddy soil, respectively. There was a significant decrease of EC in the first four days and then slow decrease in the following 6 days. The EC values of final leachates for the all columns were almost stayed in the ranges between 0.43 and 0.53 mS/cm for paddy soil and 0.41 – 0.57 mS/cm for grass soil.

The change of EC values of sludge amended soils in leachates was linked to the type of sludge addition. For example, in case of sludge amended grass soil, BCh showed the highest EC compared to other sludge. This phenomenon can be assumed by more Ca^{2+} adding in BCh sludge which leads more cation and anion release in solution by cation exchange mechanism.

In the case of BD, BCD, BFP amended paddy soil, the EC values in five leachates run in the similar range. The Cu leaching concentration was also similar values for these samples.

The final EC in amended soil leachate was most of the time higher than the control final value, thus indicating that sludge amending influenced conductivity of the leachate.

Granular sludge amended soils

In grass soil, EC decreased in BAN amended grass soil to reach the same final value as the control. The amending did not influence the conductivity of the leachate at the equilibrium. In the case of BAE, EC was higher than the control since the first leachate and then stayed stable. BAE amending led to an increase of the conductivity.

In paddy soil, EC evolution was comparable to the control. In BAN amended soil EC values were comparable to the control while, surprisingly, in BAE amended soils, EC was lower than in control soil.



- **Correlation between EC variation and Cu leaching efficiency.**

The impact of EC value at the equilibrium on copper leaching efficiency was represented on Figure 72. For both paddy soil and grass soil, increasing copper leaching efficiency was linked to a final slight increase of EC compare to the control soil. Figure 72 also represented copper concentration in the last leachate according to EC.

In paddy soils copper concentration in final leachate were closed to 0 $\mu\text{g/L}$, excepted for BD, BFP and BC for which concentrations were lower than 10 $\mu\text{g/L}$, but can partly explain higher EC in final leachate.

In the case of grass soil, all the sample leachate EC values were all higher than the control, but concentrated around 0.5 $\mu\text{g/L}$ whatever copper concentration in the leachate, thus confirming that other ionic species were present in leachate.

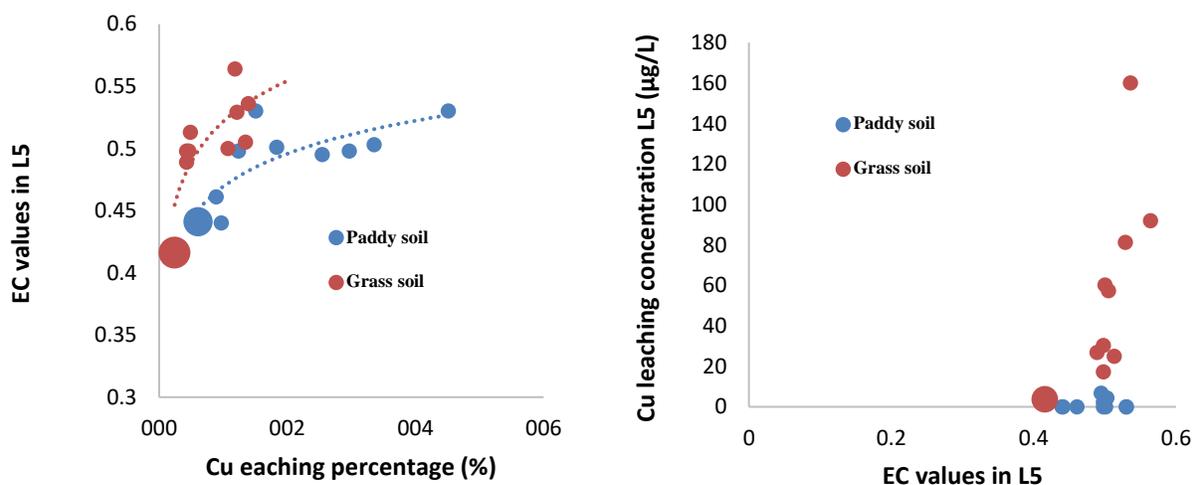


Figure 72 : Correlation between EC variation and Cu leaching efficiency and copper leaching concentration in last leachates

The effect of soil origin on EC variations and copper leaching was investigated for centrifuged sludge in Figure 72 and Figure 73. As for pH, copper leaching was not clearly associated with EC variation in paddy soil while it is the case for grass soil. In reality, if Figure 72 demonstrates a global EC increase with leaching ability, for a given sample EC decreased along time. EC decrease during the leaching process was linked with the decrease of copper concentration in the leachates. This result is in accordance with previous results (Chen et al., 2010; Fan et al., 2011).



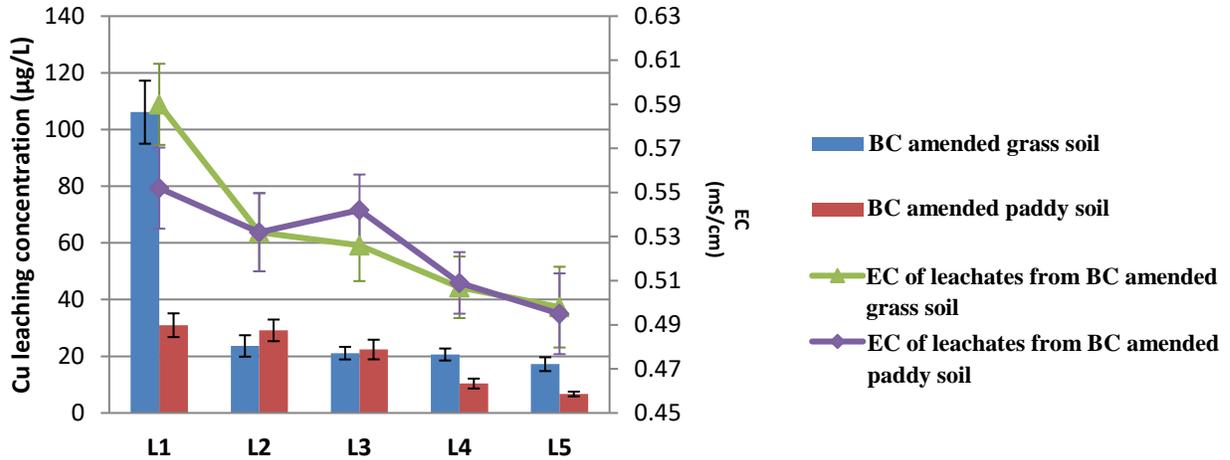


Figure 73 : The correlation between EC variation and Cu leaching concentration on BC amended soils.

- The impact of EC on Cu leaching dynamic.

As mentioned before, the final EC in amended soil leachate was most of the time higher than the control final value, thus indicating that sludge amending influenced conductivity of the leachate. It is especially true in grass soil.

This result means that the high EC value in grass soil was not only due to Cu leaching but also to other ionic species release. This is in accordance with the above mentioned results about pH variation in grass soil.

The trend of EC values was opposite with pH tendency in leachates of sludge amended soil columns. It could be hypothesized that at low pH values, the soil solution contained large H^+ ion concentration, the proton exchange occurred and metal ion elution will increase the EC values in leaching solution. In contrast, at high pH values, more metal ions can precipitated with OH^- or complex with negative ions in soil surface, thus reducing Cu leaching ability and EC values in leachates. The impact of EC on Cu leaching mechanism can be summarized by Figure 74.

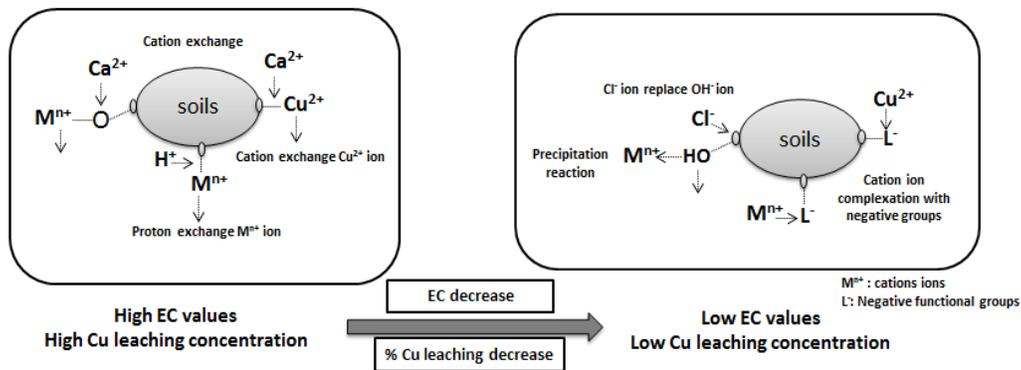


Figure 74 : Summary of correlation between EC values and Cu leaching ability during leaching process.



III.3.3. The DOC changes in leachates

Dissolved organic carbon (DOC) is a very important factor which plays an important role in facilitating the leaching of Cu in soils. DOC can facilitate Cu transport in soil and groundwater by acting as a carrier through formation of soluble metal – organic complexes (Xu et al., 2005). Figure 76 showed the evolution of DOC concentration in different leachate when sludge amended soil column. Copper, in particular, is known to have high affinity with DOC and generally reacts with DOC by forming stable coordination complexes rather than by forming compounds via hydrogen bonding or electrostatic attraction because of its low hydration energy and low charge (Fan et al., 2011).

- **DOC of control soil leachates**

The DOC showed the highest values in the first leachate (L1), then decreased quickly in second leachates (L2) and kept stable values until the end of leaching processes for both control grass soil and paddy soil. Leachates from control grass soil columns always contain higher DOC than leachates collected from control paddy soil column. This due to higher OM concentration in grass soil, compared to paddy soil. During leaching process, larger amount of OM component in grass soil can dissolve and leach.

- **DOC of sludge amended soil leachates**

CAS amended soils

In sludge amended soils (grass soil and paddy soil) the DOC concentration decreased with time. DOC in leachates rapidly decreased in first 4 days and then reached a plateau until the end of the leaching period. This trend is comparable to those of the control.

For grass soil columns, excepted LR, Cp1 and Cp2 amended soils, all the DOC at the end of the leaching process were higher than the control, thus proving that amending increase DOC in soils leachate. DOC concentration decreased rapidly since the beginning of the leaching process. This decrease is closely linked to copper desorption measured in paragraph. It could be assumed that the formation of complex DOC/Cu enhanced Cu leaching. BCh, BCD, BFP and LR have higher DOC content than other CAS sludge (See chapter 1-Figure 42). That could explain higher DOC release and thus more Cu leaching efficiency compared to other CAS sludge application.

For LR, Cp1 and Cp2 the high level of mineralization organic matter may explain the low level of dissolved DOC, for these samples, Cu complexation with DOC is probably not the main driving parameter of leaching. It also explains the lower leaching percentage in these samples.

For paddy soil columns, the similar trend was seen for all sludge amended soil samples. This trend was also comparable to the control. A rapid decrease of DOC concentration from L1 to L2 and then DOC concentration remained stable until the end of the leaching process. In leachate L1, DOC values for all the



samples were higher compare to control soil but at the end of the leaching process, DOC values were comparable to control soil. DOC was rapidly leached from paddy soil. DOC values obtained in paddy soil leachates were lower than DOC concentration in leachates collected from grass soil columns. This result is in accordance with soil characteristic; DOC is very low in paddy soil.

As far as high percentages of Cu desorption was found in first leachates (L1) and low in others, it could be hypotheses that DOC/Cu complex formation explain high leaching performance.

Granular sludge amended soils

In grass soil BAE and BAN, DOC evolution along time was quite comparable. The DOC values in leachates from granular sludge amended grass soil are always higher than the control. In grass soil the release of DOC is slower compare to the control.

In paddy soil, BAE and BAN, DOC evolutions were comparable, but the kinetics were completely different from grass soil. The DOC of BAE and BAN was rapidly leached and reached comparable value to the control at the end of the experiment.

- **Influence of soil origin.**

The evolution of DOC concentration and copper leaching was compared for a same sample for the two different soils: in the case of paddy soil, the decrease of DOC values along time clearly corresponded to Cu leaching in soil solutions, but in the case of grass soil, DOC is still decreasing while copper leaching remained stable.

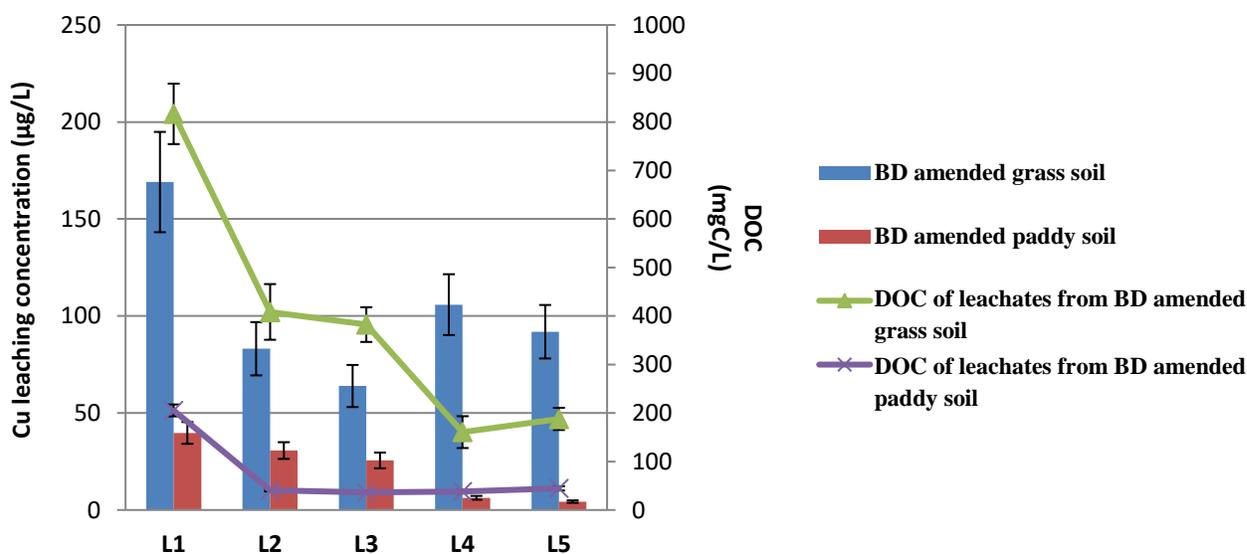
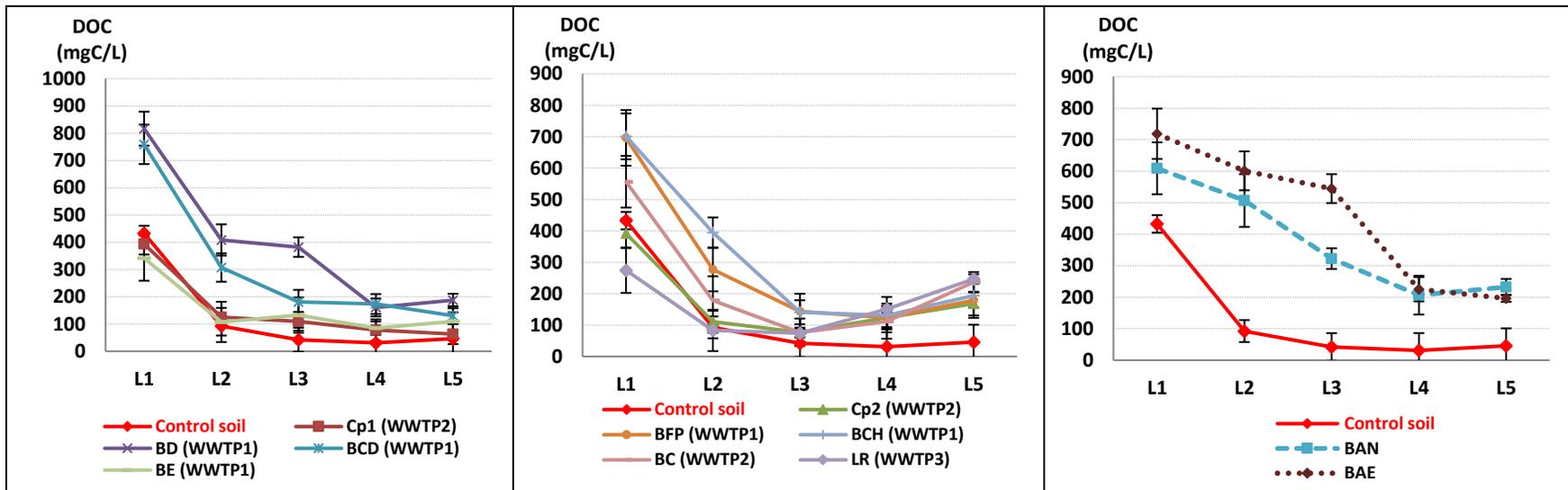


Figure 75 : Correlation between DOC values and Cu leaching dynamic in different leachates



Sludge amended grass soil



Sludge amended paddy soil

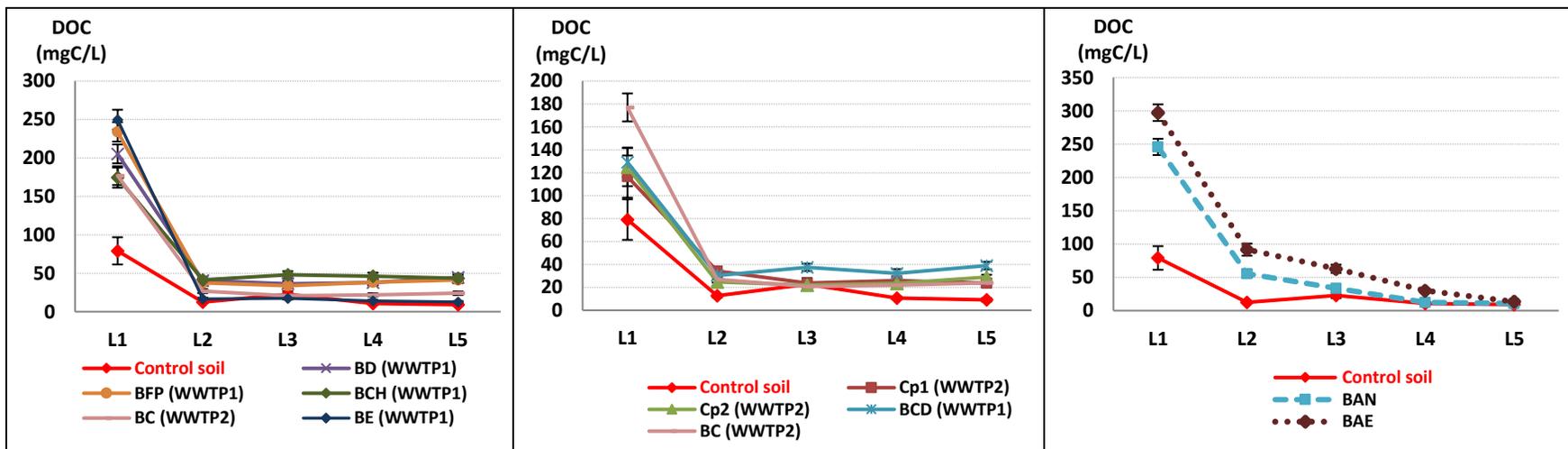


Figure 76 : Evolution of DOC in different leachates according time



- **The impact of DOC on Cu leaching dynamic.**

The reduction in Cu desorption percentages along the time was possibly due to the decrease of DOC concentration in these leachates. Besides, during leaching process, the pH increased according to the time and tended to alkaline conditions. It could be hypothesis that for these pH values from 8.0 to 10, the bonds that hold organic matter to Cu disrupted and acidic components of organic matter are converted to their soluble salt forms. Soluble organics thus increase the carrying capacity of soil solutions for metals. The increases of DOM solubilizing/mobilizing potentials at high pH values can facilitate Cu transport in soil.

As an example, limed added sludge had high pH values (around pH 12). At this pH value, the heavy metal leaching ability was often reduced when limed sludge was applied on soils (T. Pardo et al., 2014b). However, according to several researches, Cu mobility and availability in soil are mainly controlled by soil organic matter and are less pH dependent than those of other metal elements like Cd or Zn (T. Pardo et al., 2014b). According to Pardo et al., (2011b), pH higher than 6.2, increased Cu concentration linked to soil organic matter. In our study, the DOC leaching from BCh amended soils was higher than that of other conventional sludge. At high pH values DOC easily complex with Cu and leaching through soil columns.

Therefore, as Cu mobility is related to the DOC concentration in leachates, it could be assumed that DOC acted in two different steps: 1) high DOC leaching at beginning leads to more important Cu mobility, 2) lower DOC concentration may explain lower copper release.

III.4. 3D fluorescence of the leachate.

According to the lecture review, DOC-complexed Cu is more mobile in soil than simple Cu under leaching condition. DOM consists of different structures and molecular weights of organic substances such as free amino acids, carbohydrates, organic acids, enzymes, amino sugar, polyphenol, humic substances (Kalbitz et al., 2000), which can affect the bioavailability and the transferability of Cu. Moreover, DOM contains a variety of different organic functional groups such as carboxyl (COOH), phenols (OH), thiols (SH) and amines (NH₂) (Smith et al., 2002). Cu can react with the organic ligands of organic matter that directly affect their form, distribution, bio toxicity migration and transformation (Yamashita and Jaffé, 2008). DOC composition was investigated with 3D fluorescence spectroscopy.

Three dimensional liquid-phase fluorescence spectroscopy - excitation/emission matrix (EEM) fluorescence spectroscopy has been widely used to describe dissolved organic matter (DOM) composition in solution (Henderson et al., 2009). It enables the visualization of proteins (Mayer et al., 1999), humic substances (Sierra et al., 2005) and other DOM components (Dwyer and Lant, 2008). Fluorescence spectroscopy is also a sensitive method for characterizing metal complexation by DOM in natural waters or in soil, which is also considered to be one of the most promising and productive methods to analyze the impact of different DOM-ligand on the stability of Cu during leaching (Guo et al., 2012). Specific zones according to EEM wavelength were indicated as Chen et al (2003) and were described in Part 2-II.2.6 (page 92).



Table 34 : Different excitation/emission wavelength correspond with region of components

Main components	Zone	Excitation wavelength (nm)	Emission wavelength (nm)	Components
Proteinlike substances	I	200-250	250-300	Tyrosine (Tyr)
	II	200-250	330-380	Tryptophan (Try)
	IV	>250	250 - 380	Sub-microbialproteinproducts
Humic – like substances	III	200-250	>380	Fulvicacid (FA)
	V	>250	>380	Humicacid (HA)

All leachates collected from control soil and sludge amended soil columns from L1 (after 2 days) to L5 (after 10 days) were analyzed. To obtain clearly specific zone for each fluorochrome, the leachates were diluted so that absorbance at 254 nm was lower than 0.2.

The 3D-fluorescence spectra were marked as follow: the name of sample – the order of leachates – the dilution factor (*GS-L1-D5*: leachates L1 of grass control soil, diluted 5 times; *PS-Cp2-L1-D5*: Leachate L1 of composted sludge (Cp2) amended paddy soil at 5 times of dilution, for example).

- **Control soil**

Figure 77 presented the 3D fluorescence spectra of five leachates from control paddy soil and grass soil columns. At the early stages, paddy soil and grass soil fluorescence fingerprints revealed the occurrence of proteins and humic substances like. There were peaks in the five zones in the first leachate-L1.

Following the leaching process of control grass soil column, the 3D fluorescence spectra specific zones were changed. Specific zones III and V corresponding to humic substance disappeared from leachates L2 and L5, while specific zone I, II and IV corresponding to proteins maintained in spectra of leachates until 8 days (L4). Only zone II was slightly visible in leachates at the final stage of leaching process.

In case of control paddy soil column, specific zones I, IV, and V disappeared from L1 to L2. During leaching process of paddy soil column, fulvic acid (zone III) and tryptophan (zone II) and the associated DOM components were maintained in leachates. The presence of five EEM zones in leachate L1 can explain the highest Cu desorption concentration (Figure 65). It could be hypothesized that Cu was leached due to complexation with ligand like functional groups of protein and humic substances of DOM. When the content of these components reduced, the Cu cumulative concentration in leachates also decreased along leaching procedure.



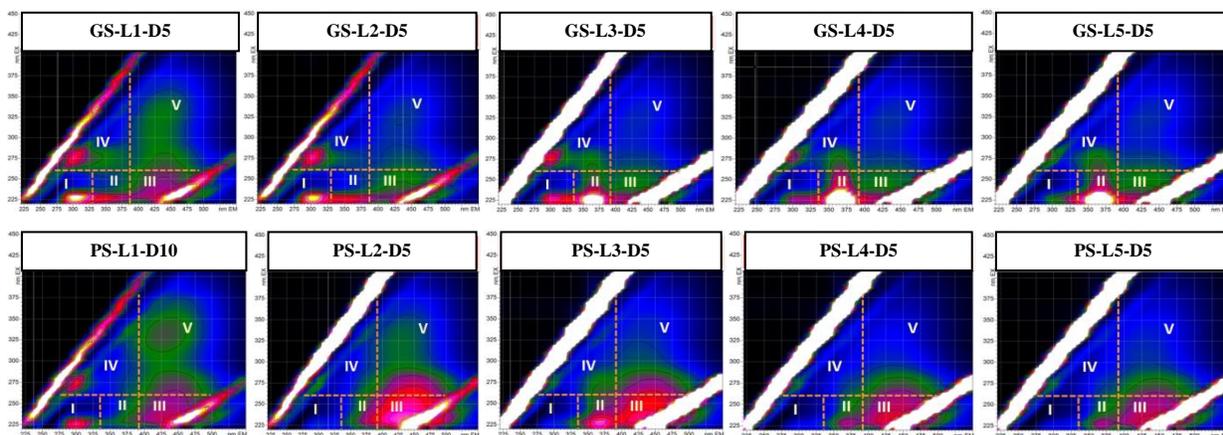


Figure 77 : 3D fluorescence spectra of leachates from control paddy soil and grass soil columns

- **Conventional sludge amended soil**

Sludge amended paddy soil and grass soil, 3D fluorescence spectra were modified following sludge amendment. Similar with first leachates of control soil, five specific zones appeared on EEM spectra whatever the kinds of sludge landspreaded. Besides, the zone II and zone IV were often presented in EEM spectra of leachates at beginning of leaching process. Then they disappeared in EEM spectra of last leachates according to the sludge amendment. These zones were characterized as Tyr and sub-microbial protein –like fluorescence which is recognized as simple DOM.

Sludge amended grass soil columns

The 3D fluorescence spectra are presented in Appendices 3

Tyr-like protein fluorescence (Zone I) and sub-microbial-like protein fluorescence (Zone IV) appeared on EEM spectra of first leachates (L1) or in some second leachate (L2) of BFP, BCh, BC and BD and then were not detected on EEM spectra of the last leachates. Some complex DOM structures such as Fulvic-like humic substances (zone III), Humic acid (zone V) almost appeared in the last leachates, together with Tyr-like protein. Comparing EEM spectra with copper leaching along time it could be assumed that at the beginning of the leaching process simple protein components of DOM easily created complexation with Cu and leached through the soil column. Indeed, we found the highest Cu only in the first leachates of sludge amended grass soil while other leachates presented low Cu leaching ability.

These results were consistent with those previously reported in the literature dealing with the evolution of fluorescence fingerprints of (Guo et al., 2012). They indicated that Cu interacted with the ligands of fluorescent Tyr –like protein. They also found that no systematic trend of the change of fluorescence intensity value of humic-like substances or fulvic-substances during Cu interaction. Their result also indicated that there was no evidence of Cu binding with humic or fulvic like acids during Cu leaching process.



The competing effect of cation Ca^{2+} for the complexation of metal Cu ions with fulvic acid has been reported (Guo et al., 2012; Wu et al., 2012). Ca^{2+} or Mg^{2+} can significantly interact humic like acid fluorescence thus explaining why humic and fulvic acids are maintained in the leachates while the Cu leaching ability and the zone of fluorescent Tyr-like protein decreased in case of sludge amended grass soils.

Sludge amended paddy soil columns

The 3D fluorescence spectra are presented in Appendices 3

According to the EEM – spectra, the five zones are not systematically present in sludge amended paddy soil. The specific zone I and zone IV appeared in leachates L1, L2 and L3 for all leachates of sludge amended paddy soil, excepted BCh, BC, BD, BFP. Compared to the control paddy soil, sludge amendment increases the humic like substances content in DOM components. The occurrence of Tyr and sub-protein like substances in leachates L1, L2 and L3 can explain the higher Cu desorption percentages compare to leachate L4 and L5. Like for sludge amended on grass soil, the humic and fulvic-like substances did not have any effect on Cu leaching ability. Instead, simple organic matter structures such as Tyr have effect on Cu mobility on soil column after sludge amendment.

- **Granular sludge amended soils**

3D fluorescence spectra of leachates from granular sludge amended soil columns were presented in Figure 78.

Granular sludge amended on grass soil

Granular sludge amended grass soil presented different results compared to conventional sludge application. The five specific zone appeared on spectra of the five leachates of BAE amended grass soil and in the first three leachates L1, L2 and L3 of BAN amended grass soil. In comparison, the five specific zones were observed only in first leachates of control grass soil and conventional sludge amendment. The possible complexation of Cu with simple and complex DOM components can explain the more important Cu ability obtained following granular sludge application (Figure 78)

Granular sludge amended paddy soil

The differences of 3D fluorescence spectra of granules application on paddy soil were drawn for BAN and BAE sludge amendment (Figure 78).

- BAN amended paddy soil: only three zones I, II, IV appeared in spectra of leachate L1 and zone II, II, V were visible in spectra of leachate from L2 to L5.
- BAE amended paddy soil : the five specific zones were detected on spectra of leachate L1, L2 and only specific zone II, III, V were found on spectra of leachates L3, L4 and L5.



In this case of granular sludge, more simple Tyr and sub-microbial protein of DOM components in leachates can be explained the more important Cu mobility of BAE amended paddy soil column compare to Cu mobility of control soil and BAN amendment.

EEM – spectra of granular sludge amendment soils did not present all five zones in all leachates, this indicates. Hence, it could be hypothesis that the granular sludge could have lower Cu mobility due to the absence of some Cu complexation with protein –like components during leaching process, compared to BCh, BFP and BD sludge amendment.

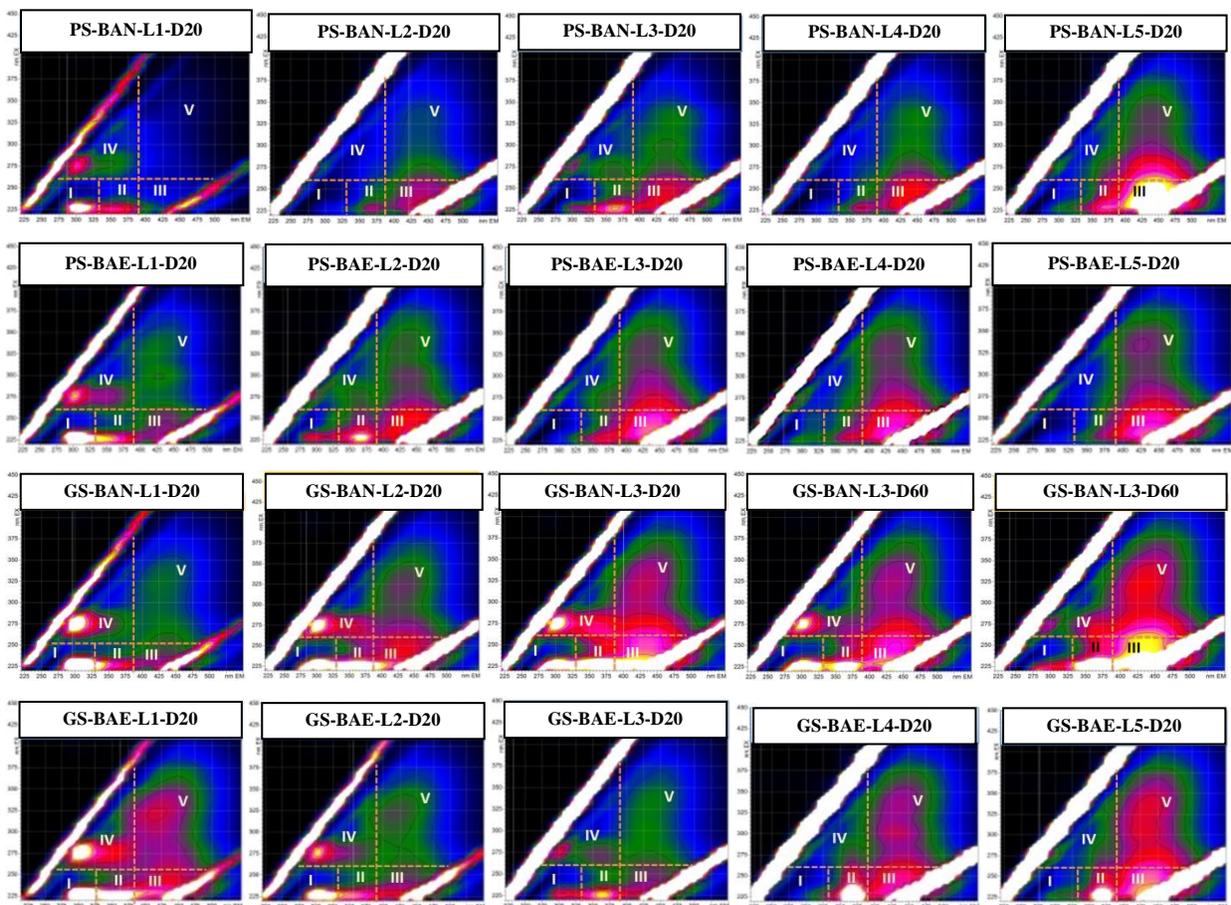


Figure 78 : 3D fluorescence spectra of leachates from granular sludge amended soil columns.

- **Summary the impact of DOM components on Cu leaching dynamic by observation on EEM-spectra.**

High amount of Cu leaching coincided with leachates exhibiting 3D fluorescence in the five zones. Such correspondence confirms the hypothesis that the dissolved organic matter component is involved in Cu transport through soil column. The DOM components release from the soil and sludge amended soil is hence controlling Cu mobility. HA and FA can be intimately related to DOM composition, leading to high binding capacity with Cu and thus more Cu mobility (Plaza et al., 2005). However, the study of Pontoni et al., (2016b) also indicated that FA had poor or no interactions with Cu during leaching on soil



column. Their study found that the dispersed release of these DOM components confirms no Cu complex with small molecular weight ligands of DOM.

In this study, it was evident that, at least qualitatively, the relative intensities within the described excitation-emission regions were changing during the experimental time. At the beginning, peak of zones I, II and IV were more abundant in EEM-spectra of leachates. Conversely, at the end of the experiment, the peak of these zones shows very low excitation-emission intensity, despite disappearance in some case of sludge amended grass soil (Cp2, LR, BD) and in almost cases of sludge amended paddy soil. This mean that the release of organic molecules happened from the soil to the leachate, but their quality and quantity was changing over time.

The peaks corresponding to zone I, II, IV were strongly related to the transport of Cu. This confirms the hypothesis according to which the Cu aggregates with protein and by-microbial product and then leach through soil column. Inversely, the leaching of humic and fulvic like acid in the last leachates could explain lower copper leaching in grass soil due to Cu mobilization.

It is finally clear that, Cu binding with the small molecules of DOM components ease leaching in soil column. The absence or reduction contents of protein and/or by – microbial products in leachates lead to decrease the Cu mobility. The summary of correlation between DOM components which was observed by EEM-spectra and Cu leaching ability was showed in Figure 79.

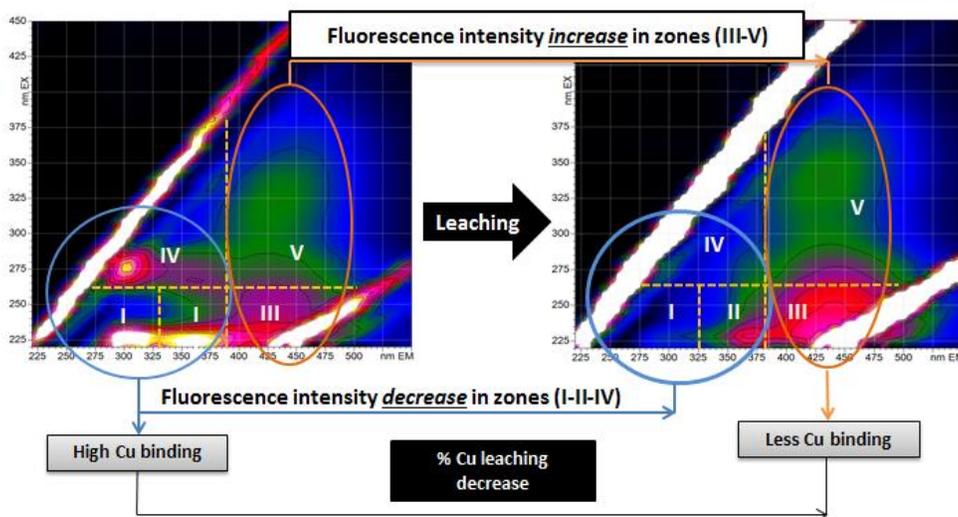


Figure 79: The correlation between Cu leaching percentage and Fluorescence intensity appearance in difference zones along time L1 and L5



III.5. Copper repartition according to the depth in amended soil columns at the end of leaching experiments

The main advantages of column leaching experiments are that they give information on current heavy metal mobility (Speir et al., 2003c). It was demonstrated in the previous part that the leaching behavior of Cu in soil column depends not only on soil incubated column but also on the kind of sludge amended.

A deeper insight in Cu distribution in soil layer can facilitate the fate of Cu in soil column.

III.5.1. Copper distribution in soil column

The concentration of Cu according to the depth was quantified following sludge application at a rate of 3 tons DS.ha⁻¹.year⁻¹ and leaching process (see previous part). The percentage of Cu distribution in different soil layers was calculated by Equation 22 (See part 2-III.3, page 111) and was presented in Figure 80.

- **Control soil:**

For the two control soils (grass and paddy soils) Cu was equally distributed in the 3 layers: around 30% in each layer.

- **Conventional sludge amended soil column**

Case of paddy soil columns

In control paddy soil column, Cu was equally distributed in each soil layer. Contrary with the grass soil, CAS sludge amending affected the Cu distribution in different soil layers. Comparing with original paddy soil, concentration of Cu was more important in the top layer compare to the bottom, suggesting that soluble copper was significantly adsorbed to soil when sludge entered into soil.

Excepted in the case of BE, sludge amendment caused an increase of Cu distribution in top soil layer from 30% to more than 40%. Cu percentage in 10 -15 cm layers decreased from 30% to less than 25% when sludge amended paddy soil.

Similarly, Buekers et al., (2007) also indicated high Cu fixation in top soil layer of five tropical Vietnamese soil due to high organic matter content after adding chemical fertilizer. Khai et al., (2007) compared the Cu distribution in the different agricultural Vietnamese soil layers by adding Chemical fertilizer, manure (chicken) and irrigation wastewater from paper industrial company. They found that Cu accumulated in the top soil layer due to adding of OM from chemical fertilizer manure and wastewater, and the amount of Cu stored in topsoil following the order of amendment wastewater > manure > chemical fertilizer, according to the reduction of organic matter.



In addition, Phuong et al., (2010b) found of higher levels of Cu accumulation (193 mg/kg) in the surface layer of paddy field compare to that of forest and upland field from Red River Delta, due to high organic matter, clay content and lower pH values of paddy soil than other soils.

Copper was highly retained in the first 0-5 cm of the column. This result can indicate that native Cu was mobilized, probably due to binding reactions with organic and inorganic ligands added with the sludge.

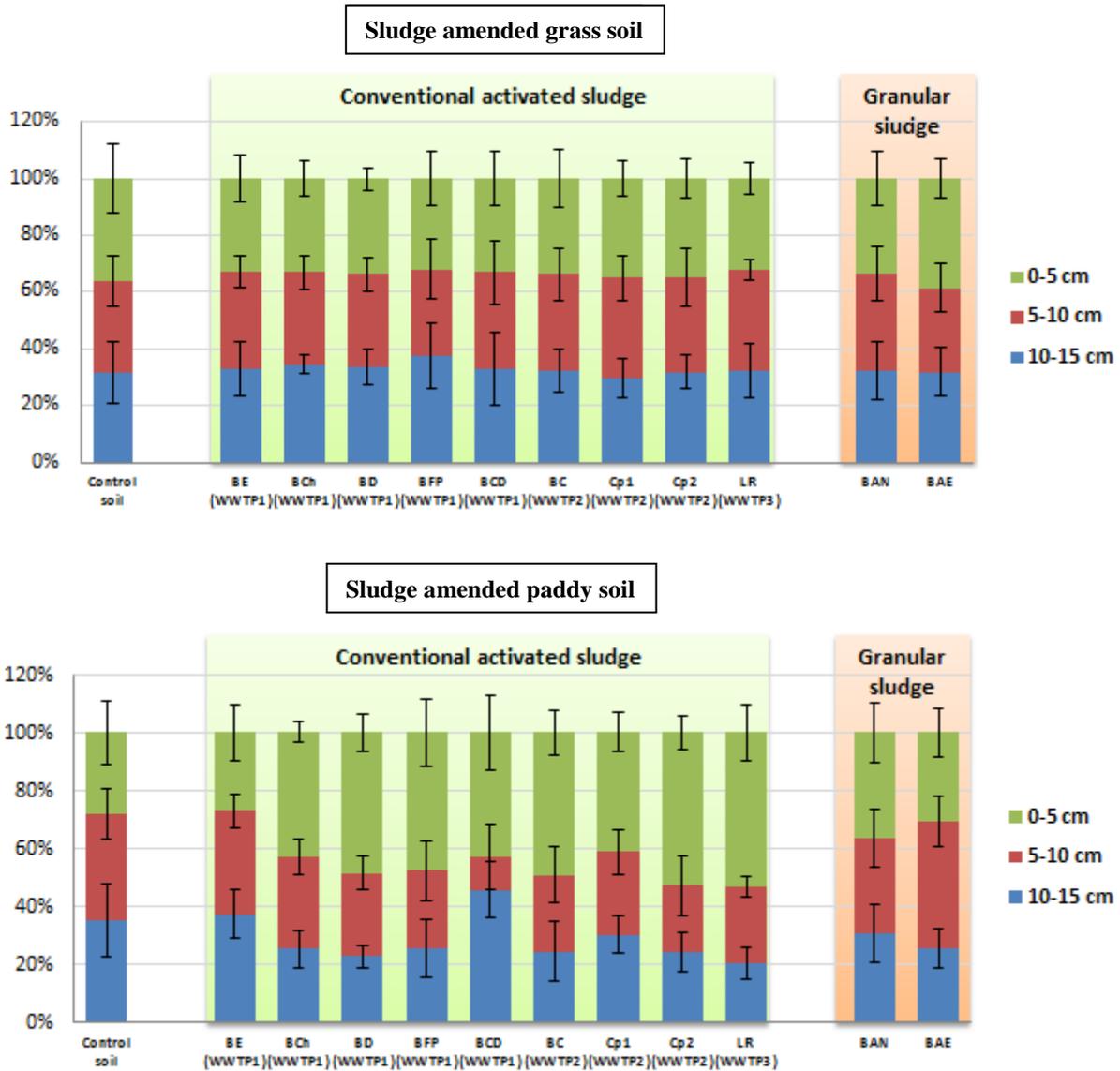


Figure 80 : Cu distribution in different layers in sludge amended soil columns after leaching experiments



Case of grass soil

At a sludge application rate of 3 tons DS ha⁻¹year⁻¹, Cu distribution in amended soils was comparable to soil control and thus was not modified by sludge amending. The Cu distribution in the different soil layer kept was quite comparable whatever the kind of sludge landspreaded: conventional or granular sludge.

- **Influence of soil characteristics:**

According to Toribio and Romany (2006d), soil with high amount of clay showed a large metal retention capacity. When sludge was added, low soluble Cu was detected in leachates, almost all the Cu was retained in the soil column after leaching experiment. Cu was equally restricted in 3 layers soil depth: 30% in each layer. As mention previously (Chapter 2 - II.2), loam-grass soil contained high clay and organic matter contents. Since clay has a very high specific surface area, it is more likely for the Cu to make bound with the clay particles resulting in Cu trapping in the soil structures (Hooda and Alloway, 1994). Moreover, clay particles can adsorb metals through ion exchange process (Peters and Shem, 1992). Besides, Cu retention has been pointed out by the binding with organic matter in soil and increasing Cu adsorption in soil structures.

Additionally, the increase of the pH values in leachates reflected neutralizing and alkali process in soil column. In this study, the soil columns were leached with artificial rainfall of CaCl₂ 0.01M. The Ca²⁺ ions have a strong acid-neutralizing capacity. It is likely that less water soluble Cu compounds such as Cu(OH)₂ were formed, other metal ions were replaced by Ca²⁺ and precipitated with hydroxide ion when pH is above 5.5 (Fan et al., 2011). These newly formed oxides and hydroxides provide additional sites for Cu sorption. Besides, pH of soil solution also greatly affect soil surface charges because of functional groups such as carboxyl, amine, hydroxyl., etc, present on the soil surface can be protonated or deprotonated. At high pH values, these functional groups on soil surface were deprotonated. Thus, as pH increase, there is an increase of negative surface charges, which can enhance the interaction and/or complexation between Cu ion and negative charges on soil surface, and reduce Cu concentration leaching in soil solution.

Moreover, the addition of sludge can result in a significant of soil organic matter and nutrient content (Suhadolc et al., 2010b). Cu adsorbed on soil via organic matter, preventing Cu movement, and explaining neckline equal amounts present in 3 layers of soil depth.

The grass soil properties (clay content, pH,..) acted to reduce the movement of Cu in soil column and kept Cu distribution stable despite sludge amendment.

- **Granular sludge amended soil**

The copper distribution was comparable to the control soil (grass or paddy soil) and comparable for BAE and BAN cu was equally distributed according to the depth of the column. Interestingly, while almost conventional sludge-amended paddy soil presented more than 40% Cu distribution in topsoil layer, granular sludge copper repartition was homogenous whatever the kind of soil approximately 30- 40% in each soil layers.



III.5.2. Copper speciation in sludge amended soils

The way copper is linked to the soil and sludge in the different soil layers after landspreading, emphasized the necessity for copper speciation according to the determination of 4 different fractions: exchangeable (F1), Fe and Mn oxides or reducible fraction (F2), organic bound or oxidizable fraction (F3) and residual (F4). These investigations using sequential extraction procedure were implemented in control soils and sludge amended soils column, after leaching processes. The results were presented on Figure 81.

Firstly, in control soils and in almost case of sludge amended soil, the distribution of copper in the different fraction can be classified as followed: the highest percentages of Cu in binding residual fractions (F4), followed by oxidizable (F3), reducible (F2) and exchangeable fraction (F1).

- **Control soil columns**

In **control grass soil**, the distribution of Cu in the four Cu fractions remained constant along the soil layers. Moreover, the proportions of Cu in F1 and F2 fractions were very low in the three soil layers. Results referring to the Cu desorption efficiency in the leachates collected from control grass soil after 10 days are reported in (Figure 63). It is possible to notice the equal Cu distribution (around 30%) in the three soil layers and similar Cu speciation in four fractions for each soil layer. Thus, it is difficult to indicate which region or fractions lead to Cu mobility in grass soil column. Slow movement of Cu at each soil layers may cause the low Cu release performance with just 0.24%.

In case of paddy soil F1 and F2 fractions did not exist in the top 10 cm soil layer, and represented only approximately 5% at the bottom of control paddy soil columns (F2). Cu fractions extracted in first and second layer (0-5 cm and 5-10 cm), were only Cu bound residual (F4) and organic matter (F3) fractions. The Cu distribution at bottom of paddy soil column was comparable to initial Cu partition in original paddy soil. This result is in accordance with copper distribution in the two first levels: in top and middle paddy soil layers there were no more labile fractions.



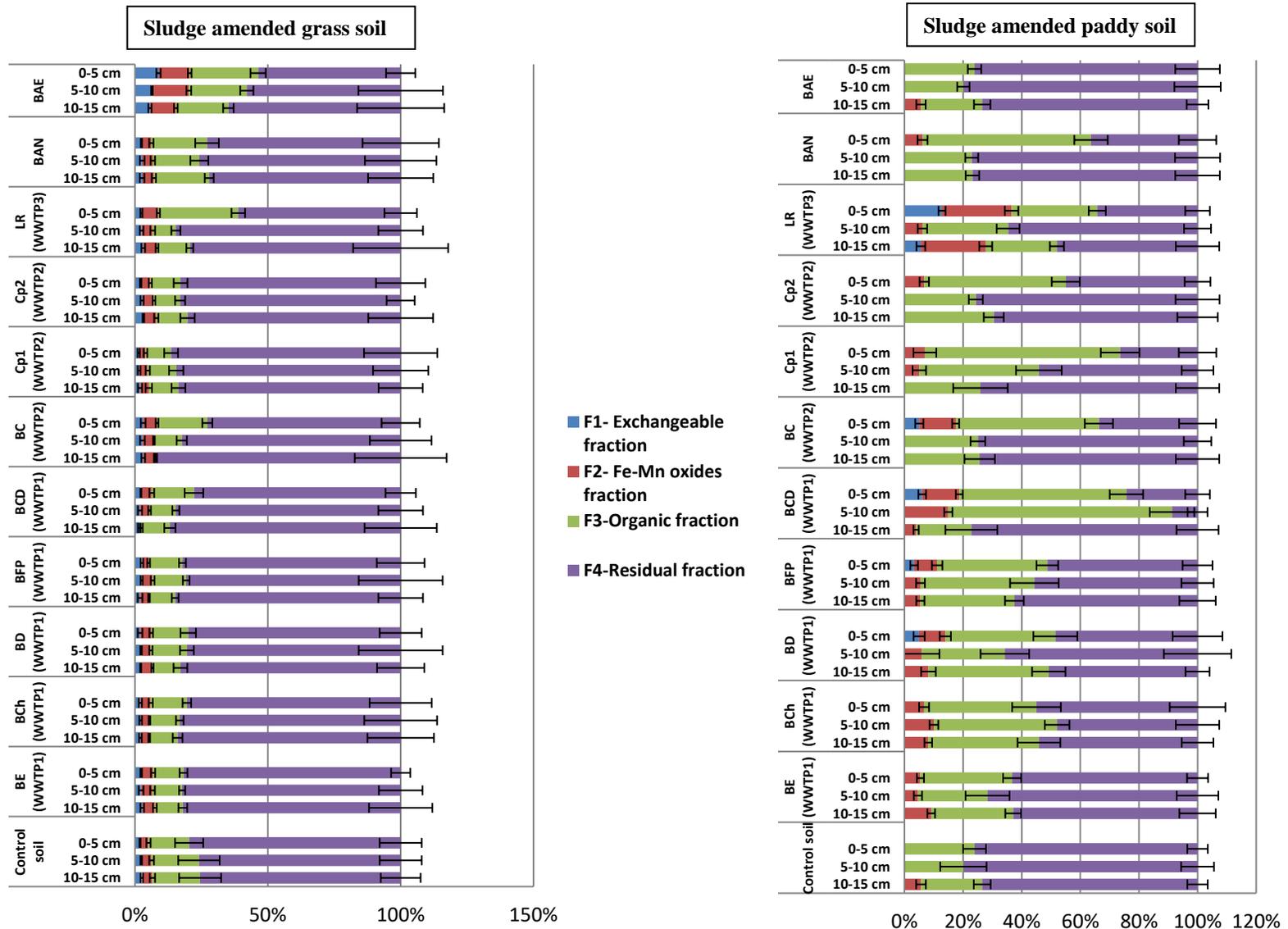


Figure 81 : Cu speciation inside the sludge column according to different layers of soil column after leaching experiments



- **Conventional sludge amended soil**

Case of grass soil: There were not clear differences in regard to copper speciation along the different layers and between soil column with and without sludge application. The percentages of Cu distribution in the different fractions remained the same in each layer of soil depth. F1 fraction corresponding to the exchangeable and water and acid-soluble Cu is very low. Probably because the copper contained in F1 was driven to deeper depth of the column and to the final leachate. The extreme weathering of the crystalline phase – F4 was the main fractions in all columns which occupied more than 70% of the total Cu in each layer before and after landspreading. Organic matter-bound Cu (F3) represented (10 – 20%) of total copper. This fraction of Cu is commonly not expected to be released in the solution. The results of copper speciation explained why Cu kept the same trend according soil depth (Figure 80) and why low percentages of Cu desorption were collected in leachates (Figure 65).

Case of paddy soil: Contrary to grass soil, paddy soil presented different trend of copper speciation according to the kind of sludge sample landspreading. It means that the organic or mineral matter brought by sludge affected directly copper speciation (and thus leaching). It could be hypothesized that in paddy soil (having very poor organic matter and clay contents), the organic matter brought by sludge amending played an important role compared to grass soil in which, organic matter was already present in bigger quantity.

The effect of sludge amended paddy soil on Cu speciation in soil layers was firstly observed on **BD, BCD, BFP, BC and LR** sludge amendment. When these sludge amended paddy soil the percentages of Cu distributed in exchangeable (F1), reducible (F2) and especially organic matter (F3) fractions increase, while Cu –bound residual (F4) fraction decreased, compared to control paddy soil and other sludge amendment. The exchangeable bound fractions – F1 is present in top of 5 cm soil depth in the range from 3.3 % (BFP) to 12.82 % (LR). Then, this fraction F1 quickly disappeared from 5 to 15 cm of soil column. Moreover, regarding to control paddy soil, Cu – bound organic matter (F3) increased significantly in the top and middle soil layers of soil columns which was amended by BCD, BFP and BC. The Cu formed in organic matter fractions ranges from around 38.85 % in the top layer of BFP to 76.33% in the middle layer of BCD sludge amendment. In contrast, the proportion of residual – F4 fraction of Cu increased as a function of soil depth. These results could be explained with the help of the sludge characterization achieved in chapter 1. BCD, BFP, BC and LR sludge contained not only higher organic matter and material contents but also more Cu distribution in exchangeable (F1) and oxidizable (F3) fractions than other CAS sludge. When these sludges amended paddy soil, they significantly bring more organic matter in soil, especially in the top layer, leading to more Cu binding with organic component and redistribution in soil layers. Furthermore, the increase of Cu bound to mobile fraction (F1, F2) may cause more Cu leaching ability compared to CAS sludge amendment.

In addition, **BCh** landspreading soil columns exhibited high Cu proportion in F3 fraction (around 40% to 50%). BCh sludge characterization (chapter 1) indicated high DOC contents. In BCh amended soil, the DOC components can be assumed to play two roles:



- On one hand, DOC can bind with Cu for leaching dynamic through the soil column. Such soluble organic matter complexes could account for the elevated Cu found in leachates, as these are likely to be the most mobile forms of Cu in soils (Speir et al., 2003b).
- On the other hand, DOC acted like a bridge for connection and interaction between cation such as Cu and soil. Organic matter was generally considered for high capacity of Cu adsorption.

Thus, the amendment of BCh in paddy soil could be explained by both an increase of Cu distribution in oxidizable (F3) fraction and/or Cu desorption percentages in leachates (4.51%).

Composted sludge: In the previous part, in case of Cp1 and Cp2 amending, it was demonstrated that Cu stored in the top 5 cm of soil depth. The percentages were equal to 41.19 % and 51.82 %, for Cp2 and Cp1 sludge sample, respectively. Cu extracted in this layer was mainly distributed in the Cu bound to oxidizable fraction (F3) compared to other fractions. The percentage of Cu associated to Cp2 and Cp1 was equal to 48.27 % (Cp2 amendment) and 66.61 % (Cp1 amendment) respectively. The percentages of Cu in F3 fraction reduced in 10 and 15 cm soil layers. The distribution of Cu in these layers was following this order: $F4 > F3 > F2$. There was no Cu in F1 in any soil layer of composted sludge amendment. The high amount of Cu bound inoxidizable (F3) fraction, in case of composted sludge application, could be explained by the composting process itself. The high binding of Cu on stable organic fraction in top soil layer of composted sludge amendment could explain lower Cu desorption efficiency compared to other CAS sludge. Comparable results were obtained in paddy soil irrigated by wastewater from the Red River Delta, Vietnam (Marcussen et al., 2008). They showed that Cu content reduced from topsoil to the subsoil layer and that high amount of Cu is bound to Fe-Mn oxides and organic matter. The order distribution of Cu followed this order: $F3 > F4 > F2 > F1$ in the top (0-25 cm). They indicated that the addition of wastewater increased Cu binding to organic matter.

BE: in the case of BE amended on both grass soil and paddy soil, the Cu distribution in different fractions of the three soil layers maintain quite stable, comparing between BE amended soils and control soils. Suhadolc et al., (2010b) demonstrated that in the case of the application of thickened sludge to loam soil, sludge amendment caused an increase of total concentration of Cu in the upper 2 cm soil layer at sampling date. Their results of sequential chemical fraction indicated that the sludge amendment significantly increased the content of the less available Cu forms and Cu distribution did not significantly change between different soil layer.

CAS amended paddy soil led to an increase of Cu bound organic matter fraction (F3) a long soil layers compared to control paddy soil and sludge amended grass soil. Paddy soil is sandy loam soil and has very low organic matter and large size of particles. So during leaching process, the organic matter can move more easily in paddy soil column compare to grass soil column. The transportation of organic matters, after sludge amendment, may contribute to Cu bound-organic matter fractions redistribution in each soil layer and thus affect Cu leaching ability.



- **Granular sludge amendment.**

BAE landspreading on grass soil significantly increased the content of active Cu forms due to more Cu bound-exchangeable (F1) and reducible fractions (F2), compared to control grass soil, BAN and other conventional sludge amendment. For examples, in native sludge, the percentages of Cu bound to F1 and F2 fractions in BAE were 13.97 and 5.17%, while they are only 3.97 and 1.17% in BAN, 1.18 and 3.20% in initial grass soil, respectively (See chapter 2 - II.1, page 138). After leaching process of BAE amended grass soil, the Cu distribution in F1 and F2 remained quite constant: from 11% and 12 % at the top soil layer to 5% and 7% at the bottom layer, respectively. Besides, both BAE and BAN sludge landspreading on grass soil led to increase the Cu-bound F3 fraction in each soil layer. The Cu distribution in soil after granular sludge amended grass soil could explain the high Cu leaching ability, which was around 1.83% and 2.04% when BAN and BAE amended grass soil, respectively.

In case of paddy soil, copper fractionation along the different soil layers was not drastically different from the control. When BAN granular sludge was amended on paddy soil, F3 fraction represented 57% at top soil layer, and then the Cu in this fraction decreased to around 25% at subsoil layers. A similar phenomenon on Cu distribution was observed in case of BAE amended paddy soil. Comparing with control paddy soil, we observed a slight increase in oxidizable Cu content (F3) and a general decrease in less labile of residual Cu fraction, particularly in topsoil layer.



III.6. Conclusions

The column desorption tests, were carried out on conventional and granular sludge samples and for the two soils (grass soil from France and paddy soil from Vietnam). The presented results showed that Cu leaching was depending on the kind of sludge applied and of the origin of the soil.

Moreover, the results were compared to those obtained in batch conditions (chapter 2). Most of the time batch conditions led to an over estimation of leaching ability. Nevertheless, batch tests can give a rapid prediction about copper leaching tendency but cannot be used for direct leaching percentage measurement. This is possibly due to the different in the experiment procedure (mixing turbulence for batch tests as opposed to preferential up-flow for the column tests).

Conclusions about conventional activated sludge (CAS) leaching ability

- **Higher Cu mobility** was evidenced in sludge amended **paddy soil** columns compare to sludge amended grass soil columns and whatever the kind of sludge origin (conventional or granular sludge).
- Considering a same sludge amendment, Cu leaching was more important in **paddy soil** compared to grass soil due to **lower clay content, lower soil pH, and lower organic matter contents** in paddy soil.
- Some conventional activated sludge samples like exhibited the same behavior when landspreaded either on grass soil or on paddy soil: high Cu desorption was obtained in the case of **limed sludge, filter-press sludge**, and low Cu leaching percentages were measured for **composted sludge** amendments.
- **Reed bed, centrifuged and centri-dried sludge** presented different behavior in regard to Cu desorption abilities when they were amended on paddy soil or on grass soil. The lowest Cu desorption efficiency was measured on centrifuged sludge amended grass soil (0.43 %) and on reed bed amended paddy soil (1.07 %).

Conclusions about granular sludge leaching ability

Granular sludge amended soils, tends to **release readily more Cu** than conventional sludge. Nevertheless, **Cu is less available** when granular sludge was amended **on grass soil** than when they are landspreaded on amended paddy soil. To our knowledge, there are no papers mentioning the effects of granular sludge amended soil on heavy metal leachability. Interestingly, from our results, granular sludge acted like some kinds of conventional sludge such as limed sludge, filter-pressed or thickened sludge by increasing the leaching potential of Cu when they were amended on soils. This phenomenon can be explained by many sludge characteristics such as surface functional groups, that can complex and interact with soil components for replacing Cu ions, thus increasing the Cu leachability.



Factors affecting Cu leaching:

- In most of the cases, sludge amendment led to a modification of leachate characteristics compare to non amended soil.
- During the leaching process, pH of leachates from grass soil and paddy soil columns increased while EC and RP values decreased. It could be linked to the decrease of salts and reducing condition in soil column. Moreover, the dissolved organic carbon (DOC) contents went down quickly to stable values at the end of leaching process.
- There are no significant differences in DOC concentration between sludge amended and non-amended soils at the end of leaching. A more thorough insight in dissolved organic carbon composition by using 3D-fluorescens spectroscopy allowed demonstrating that leachate biochemical composition greatly influenced Cu mobility. The occurrence of high pick intensity in the zone corresponding to protein substances such as Tyrosin and sub-microbial like protein have a positive effect on Cu leaching ability while humic and fulvic like substance effect were unobvious.

Copper repartition according to the depth (0-5 cm, 5-10 cm, 10-15 cm) in amended soil columns at the end of leaching experiments

- **Grass soil:** Cu distribution in amended soils was comparable to soil control and thus was not modified by sludge amending. The Cu distribution in the different soil layer kept was quite comparable whatever the kind of sludge landspreaded: conventional or granular sludge. The grass soil properties (clay content, pH,..) probably reduce the movement of Cu in soil column and kept Cu distribution stable despite sludge amendment.
- **In control paddy soil column,** Cu was equally distributed in each soil layer. But sludge amending affected the Cu distribution in different soil layers. Copper was highly retained in the first 0-5 cm of the column. This result can indicate that native Cu was mobilized, probably due to binding reactions with organic and inorganic ligands added with the sludge.
- **Granular sludge:** Cu distribution according to the depth was not modified: neither by the origin of the granules (BAE, BAN), nor by the soil (grass or paddy soil). The distribution was also comparable to the control soils.

Copper speciation according to the depth (0-5ccm, 5-10 cm, 10-15 cm) in amended soil columns at the end of leaching experiments

In order to have a more accurate understanding of the mechanisms driving Cu leaching, Cu speciation was determined in each soil layers.



- **Grass soil:**

CAS: There were not clear differences in regard to copper speciation along the different layers and in regard to sludge landspreading. Cu distribution in the different fractions remained the same in each layer of soil depth: F1 was very low, F4 was the main fractions in all columns and occupied more than 70 % of the total Cu. F3 represented (10 – 20%) of total copper. The results of copper speciation explained why Cu kept the same trend according soil depth.

Granular sludge: BAE landspreading on grass soil significantly increased the content of active Cu forms (F1) and (F2), compared to control grass soil, BAN and other conventional sludge amendment. Fractions F1, F2 and F3 were higher in the different layers of granular sludge compare to CAS samples. The Cu speciation in soil could explain higher Cu leaching ability of granular sludge compare to CAS samples.

- **Paddy soil:**

Conventional sludge

- Cu bound oxidization fraction (F3) increased in almost case of sludge amended paddy soil, especially in the top soil layer.
- In the case of limed and thickened sludge amended paddy soil, Cu was distributed in three fractions: reducible (F2), oxidizable (F3) and residual forms (F4) with higher percentages compared to control paddy soil.
- Filter-press, centrifuged, centri-dried, and digested and reed bed sludge exhibited not only more Cu distribution in oxidizable fraction (F3) but also higher Cu bound to exchangeable (F1) and reducible (F2) fractions in topsoil layer. The increase of active fractions leads to an increase Cu leaching ability.

Granular sludge

- Cu linked to organic matter fraction F3 was mainly localized in top soil layer in the case of granular sludge amended paddy soil. They represented 23.90 % and 57.48% in case of BAE and BAN application, respectively. This phenomenon was also observed in case of some conventional sludge amended Paddy soil such as composted sludge, centrifuged and centri-dried sludge.

The effect of sludge and soil characteristic on Cu distributions and Cu leaching availability after sludge amended grass soil and paddy soil were synthesized in Figure 82. Paddy soil is sandy loam soil and has very low organic matter and large size of particles. So during leaching process, the organic matter can move more easily in paddy soil column compare to grass soil column. The transportation of organic matters, after sludge amendment, may contribute to Cu bound-organic matter fractions redistribution in each soil layer and thus affect Cu leaching ability.



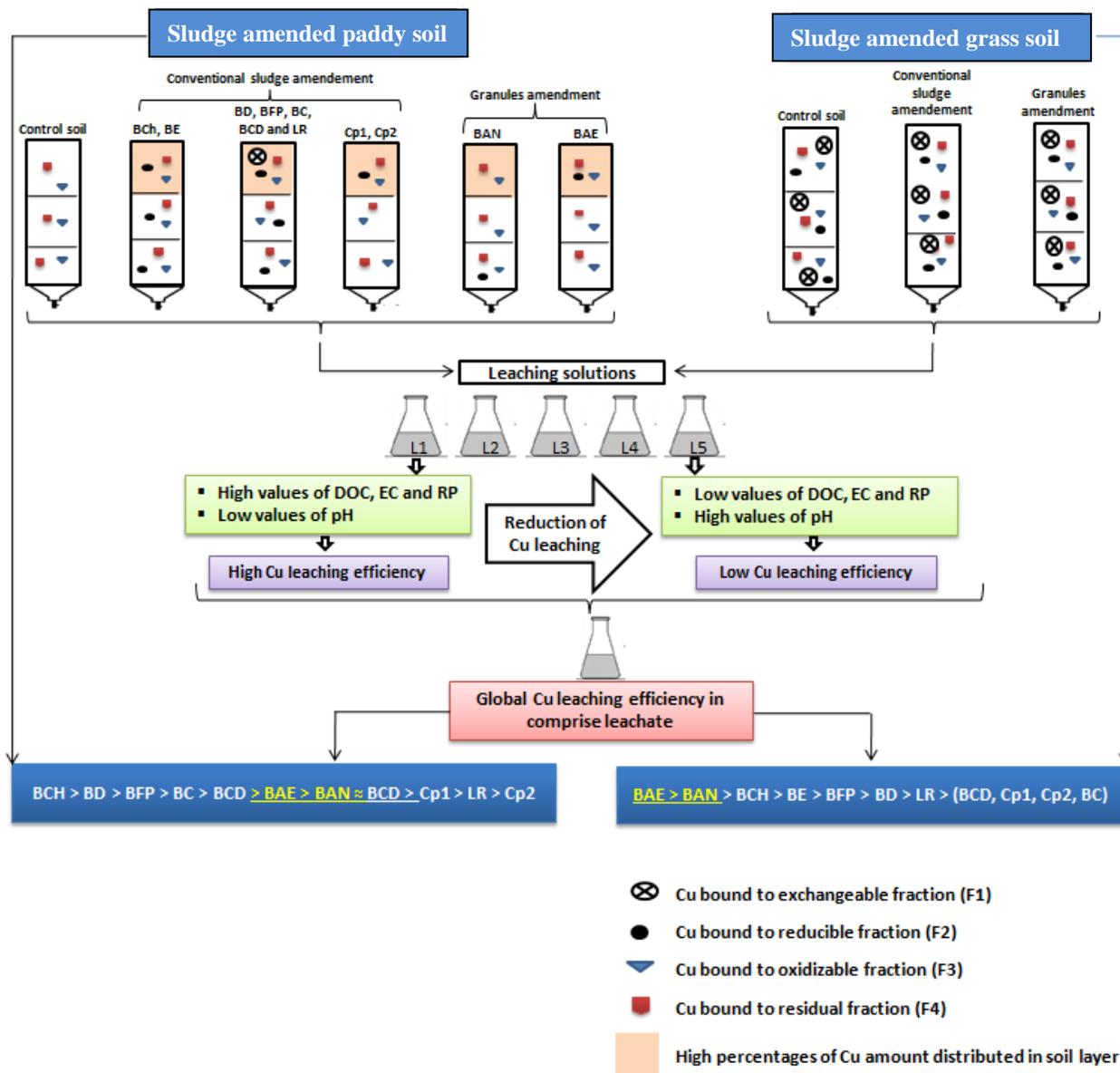


Figure 82: Summary of Cu distribution in soil column and Cu leaching percentages tendency in paddy soil and grass soil column after leaching process.

On grass soil, granular sludge led to the most important leaching ability. This tendency was noticed in both cases of experiments: batch and column.

Inversely in paddy soil, granular sludge leaching ability was low and comparable to highly stabilized CAS (composted, reed bed).

Limed, filter-pressed and digested sludge amended soil (grass and paddy soil) require warning about the risk of copper leaching potential to further agricultural activities for reusing sewage sludge.



CONCLUSIONS & PERSPECTIVES





CONCLUSIONS & PERSPECTIVES

Conclusions

The fate of heavy metal during bio-treatment of wastewater in conventional activated sludge has been widely investigated. It is also possible to find some papers about heavy metal concentrations in the new promising technology – granular sludge.

At the end of wastewater treatment, sludge is stabilized by different specific methods. These processes also affect the accumulation or mobilization of heavy metal and thus their fate during landspreading. Moreover, 75% of sludge after stabilization was valorized in land application in France, the fate of heavy metal after sludge landspreading also attracted great attention due to the leaching ability of heavy metal which can affect directly to the ground or surface water quality and therefore plant production, environment and human health.

However, the comparison of stabilization processes on the fate of heavy metal after landspreading remains limited because of the wide variety of sludge and soil origin. There is thus a lack of information about the impact of sludge stabilization processes on the fate of heavy metal during landspreading on a given soil. In the case of granular sludge, it is very difficult to find any research paper focusing on this issue.

It was proven that CAS and granular sludge treatment impacted deeply on sludge structure, composition, and on interactions with heavy metal. But to our knowledge there are no studies allowing comparing the two technologies in terms of heavy metal fate in case of sludge valorization by landspreading. Hence, it is hard to grasp the reasons for heavy metal accumulation or leaching in sludge, if only focus on certain wastewater treatments or sludge treatment stages, especially after sludge amended on soil.

The question of the choice of a wastewater treatment process (CAS, granular) is linked to technical (the kind of effluent, flow rate, loading, etc) and also to financial considerations (like energy consumption, sludge management, etc). But the choice of a process in never had done in terms of sludge ability to transfer micropollutants to the soil in case of landspreading. The results are treated separately making it impossible any comparison.

The objective of this thesis is thus to start from “the end” by making a comparison between wastewater and sludge treatment processes in terms of copper sorption and desorption ability.

For this, samples from different wastewater treatment processes (CAS and granules), and different sludge stabilization processes (liming, digesting, thickening, drying and composting) were collected.



Then the impact of soil characteristics on heavy metal leaching ability after sludge application was conducted in the context of two different countries France and Vietnam. In both cases agricultural land were chosen for lab-scale experiment. The results of study aimed to contribute to further elucidate the fate of heavy metals according to the kind of wastewater treatment, the kind of CAS process stabilization. At last it could be interesting to propose some advice on what kind of wastewater of sludge treatment is better adapted to a country (in terms of soils characteristics). The study of paddy soil (from Vietnam) will provide the useful information on heavy metal availability when the sludge from WWTP was recycled as fertilizer on land application which is very scarce and growing interest in Vietnam currently.

In this work a methodology was developed a methodology has been developed to answer to the above described scientific inquiry.

- The sludge and soil characteristic were firstly characterized. This part of the work led to a better knowledge of the sludge and soil chemical and biochemical composition. Moreover biochemical composition and Cu concentration distribution between particulate and soluble phase was investigated.
- The Cu sorption characteristics were assessed on each sludge samples and soils via adsorption modeling in batch test study.
- Then, the copper leaching ability was measured in batch conditions and in lab-scale columns for each sludge, soil and sludge mixed sample.
- The fate and speciation of copper in the different soil layers during leaching was investigated in soil column study.

The main results obtained in this work are detailed below

Sludge and soil characterization

11 sludge (9 conventional sludges and 2 granular sludges) and 2 soils (grass soil from France and paddy soil from Vietnam) were collected and then characterized (Figure 83). For sludge sample, a specific characterization was conducted in the ‘soluble’ and ‘particulate’ sludge phase separately.

The proportion and the nature of surface functional groups (proton, cation exchange capacity, pKa of the functional groups) were determined. It was thus possible to begin to think about some possible interactions with copper. For example, the determination of the functional groups revealed that sludge particulate phases were mainly composed of the carboxyl, phosphoric and amine or hydroxyl types.

In parallel, the soils were also characterized for determination of soil particular size, organic matter and clay component, cation exchange capacity and pH values. The results obtained can also give some precious information for further interpretation of the interaction between copper ion and soil.



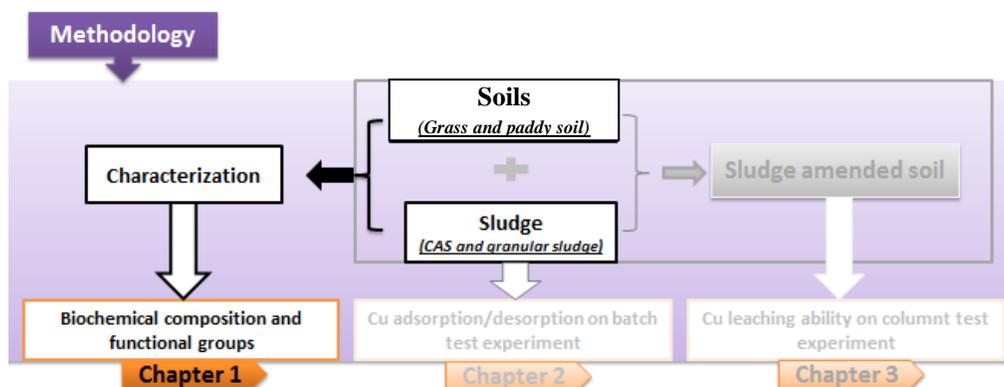


Figure 83 : Main methodology for sludge and soil characteristics

The resulted of sludge characteristics was presented in chapter 1 of result parts.

The main observation included:

- **Sludge characterization**

Granular, composted, and reed bed sludge presented high material (organic and mineral material) content and high protonic exchange capacity in particulate phase of sludge.

In Limed, filter pressed, digested thickened sludge a large protein, polysaccharide and “humic like” substances content was measured in the soluble phase while the distribution of the corresponding fluorescence intensity in the floc (observation of confocal laser scanning microscopy) was low.

Large DOC content observed in soluble phase of limed sludge, centri-dried and anaerobic granular sludge corresponded to high protein contents under the 3D-fluorescence observation.

High content of carboxyl and phosphoric functional groups was measured in particulate phase of granular, composted and dewatering sludge while they were present in high percentages in soluble phase of limed, digested, thickened sludge.

- **Soils characterization**

Grass soil contained higher CEC, organic matter and clay contain with smaller soil particular, compared to paddy soil. Both of soils are acidic soil with pH values is 5.6 and 5.2 for grass and paddy soil respectively.

Copper adsorption/desorption ability in soil and sludge alone and in soil/sludge mixing under batch conditions

In this part of study, the copper adsorption ability in sludge, soil and sludge mixed soil was determined by the determination of the constants following adsorption isotherm models. Three models were used: the



linear (K_d), the Langmuir and the Freundlich model. In parallel, the copper desorption ability was measured and expressed in terms of copper desorption efficiency. The main approaches were reported on Figure 84 and the results of study presented in chapter 2 allowed comparison between granular and conventional activated sludge before and after amendment on soils.

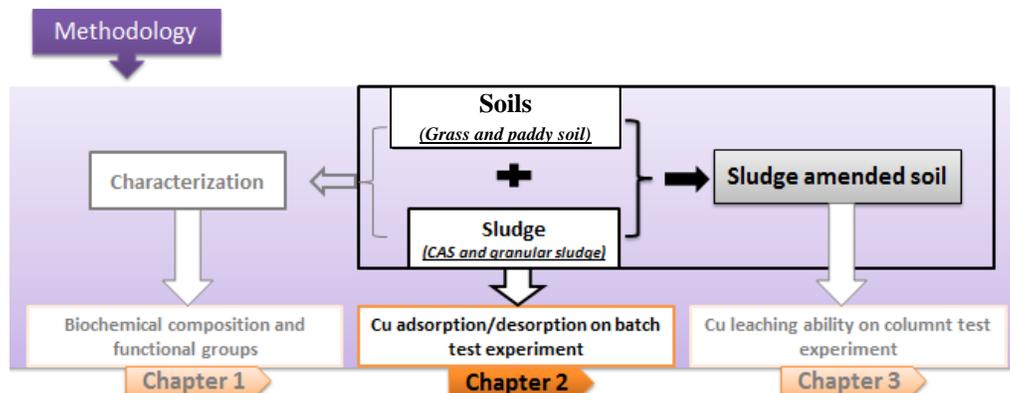


Figure 84: Main methodology for study copper availability in sludge, soil and sludge amended soil

Cu adsorption ability

Case of sludge

The K_d (linear model) values associated to conventional sludge samples varied according to the following decreasing order: Cp2 > Cp1 > BCD > LR > BD ≈ BE > BC > BFP > BCh.

The K_d of aerobic granular sludge (BAE) was higher than the K_d value of anaerobic granular sludge (BAN). Granular sludge had copper adsorption capacity comparable to composted and centri-dried sludge.

Case of soil

Grass soil-copper adsorption capacity was higher than paddy soil due to higher copper clay and organic matter content in grass soil.

Case of sludge amended soil

The only parameters which were really affected by sludge amending rate were q_{max} ; K_d and K_f for grass soil and only q_{max} for paddy soil.

The order of Cu desorption on sludge amended grass soil was: BCh > BD ≈ BE > BFP > LR > control grass soil > Cp1 ≈ Cp2 ≈ BC ≈ BCD.

- In sludge amended paddy soil the order of copper desorption percentages was: BCh > BFP > control-paddy soil > BD ≈ BC > BCD > Cp1 ≈ LR > BE > Cp2.



- Cu desorption efficiency showed higher values for aerobic granules amended soils compared to anaerobic granules.

Cu desorption ability

Case of sludge

- Low copper desorption efficiency (<5%) were measured.
- Copper was more mobile in limed and filter-pressed sludge compared to other CAS sludge due to the reduction of copper distribution in exchangeable or reducible fraction.
- Aerobic granular sludge showed high copper adsorption capacity and also high copper desorption efficiency.

Case of soil

Grass soil contained high copper retention and low copper desorption efficiency with only 0.86%, while it was 12.47% in paddy soil.

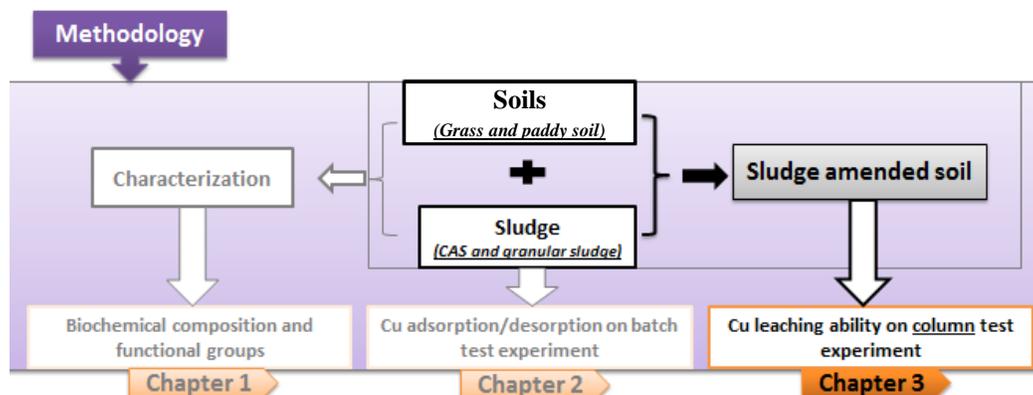
Case of sludge amended soil

- Sludge amended at high application rate of 150 tons/ha leads to increase of copper leaching ability in two soils.
- Granular sludge amended grass soil enhanced the copper leaching ability, but reduced the copper release in paddy soil.
- Limed, filter-press, centrifuged and digested sludge had more attractive for copper desorption while other sludge led to immobilized copper in soil when sludge amended on grass soil.

Copper leaching behaviors in sludge lanspreading soil column

The availability of the copper during the application of the stabilized sludge was evaluated by the use of laboratory scale soil column leaching tests. The copper leaching behavior was determined by analyzing the copper concentration in leaching solution and copper repartition in soil column after leaching tests. The column experiments were conducted on control soil and sludge amended soil with a sludge application rate of 3 tons/ha corresponding with French legislation for land application of sludge. Artificial rainfall conditions were simulated according to annual rainfall in Limousin region, France. The main significant results of copper leaching ability in soil columns were presented in Figure 85.





Case of soil

Higher copper leaching was obtained in paddy soil compared grass soil column with and without sludge application.

Case of CAS: Whatever the soil origin composted (Cp2 and Cp1), centri-dried (BCD), centrifuged (BC), reed-bed (LR) sludge presented low Cu release (lower than 2%). In contrast, Filter-pressed (BFP), digested (BD), thickened (BE) and limed (BCh) sludge amended soils increased Cu availability in soil

Case of granular sludge: The copper leaching on granular sludge amended grass soil was often higher than those measured for conventional sludge samples. In the case of sludge BAN and BAE amended paddy soil, leaching percentage was slightly higher than some conventional sludge samples amendment such as composted (Cp1 and Cp2), thickened (BE), reed bed (LR) and centri-dried sludge (BCD), but lower than limed (BCh), filter-press (BFP), digested (BD) and centrifuged (BC) sludge on Cu leaching percentages. Thus granular sludge landspreading limit desorption in paddy soils.

Batch conditions can give be used for a rapid prediction about copper leaching ability tendency but cannot be used for direct leaching percentage measurement. This is possibly due to the different in the experiment procedure (over end tumbling for batch tests as opposed to preferential up-flow for the column tests).

In the case of granular sludge, batch tests led to an over estimation of copper leaching ability, especially in the case of BAE and cannot even be used to draw a tendency.

Figure 85: Methodology and main results obtained for Cu availability on sludge amended soil column study.

Factors affecting copper mobility

pH: Grass soil leaching efficiencies covered a lower range compare to paddy soil. Grass soil, pH increase was more or less linked to an increase of copper leaching. But in the case of paddy soil, it was not possible to establish any correlation: pH values stayed constant (around 5) whatever the copper leaching percentage.



EC: The final EC in amended soil leachate was most of the time higher than the control final value, thus indicating that sludge amending influenced conductivity of the leachate. The high EC value in grass soil was not only due to Cu leaching but also to other ionic species release. This is in accordance with the above mentioned results about pH variation in grass soil.

The trend of EC values was opposite with pH tendency in leachates of sludge amended soil columns. It could be hypothesized that at low pH values, the soil solution contained large H^+ ion concentration, the proton exchange occurred and metal ion elution will increase the EC values in leaching solution. In contrast, at high pH values, more metal ions can precipitated with OH^- or complex with negative ions in soil surface, thus reducing Cu leaching ability and EC values in leachates.

DOC: Cu mobility is related to the DOC concentration in leachates, it could be assumed that DOC acted in two different steps: 1) high DOC leaching at beginning leads to more important Cu mobility, 2) lower DOC concentration may explain lower copper release.

3D fluorescence spectra of the soluble phase showed that Cu was probably binding with the small molecules of DOM components and thus ease leaching in soil column. The absence or reduction of protein and/or by – microbial products contents in leachates lead to decrease the Cu mobility.

Cu leaching mechanism on sludge amended soil column proposal

To explain the leaching behavior of Cu after sludge application on soils it was assumed to use a mobilization mechanisms based on the main physical model including: 1) an immobile phase, 2) a slowly mobile phase, 3) mobilizable phases (Pontoni et al., 2016b)

The **slowly mobile** phase model pointed out that the movement of heavy metal was controlled by colloids which were organic and inorganic, as well as macromolecular aggregates of clay components. During transportation, heavy metal passed necessarily through two regions which were separated by gradient of colloids concentrations, under the driving of leaching solution flow ($CaCl_2$ 0.01M). This phenomenon will affect to the adsorption/desorption of Cu during the movement through the soil column. Colloids in the region with low concentration often stay in the top soil layer while high colloids concentration normally stored at the bottom of column tend to adsorb Cu and reduce Cu mobility (Figure 86). This model could explain the low ability of Cu release from grass soil column which contain high amount of OM, clay content and low soil particular size. Moreover, when composted, centrifuged and centrifuged sludge were added on grass soil, the nature of organic matter differed from other samples, leading to higher Cu adsorption capacity and reducing Cu mobility. A similar phenomenon was observed when composted sludge was amended on paddy soil.

The **immobile phase model** indicates strong interactions between heavy metals and soil components, especially when sludge amendment. The use of this model implies that Cu stayed in high concentration in the top layer of soils and in low concentration in the subsoil or bottom layers of soil with and without



sludge amendment. However, some part of immobile phase can become mobile phase due to the degradation of organic matter, reduction of Fe-Mn hydro (oxides) or mineralization. This leads to create high mobile and low molecular weight organic matter. With the transition of leaching solution, these molecules will interact as ligands with Cu ions by proton/cation exchange or complexation, etc and promote Cu leaching ability. The degradation of immobile component supports the increase of the pH values, the decrease of DOC, EC and RP values during leaching process and decrease of Cu leaching dynamic in soil column (Figure 86).

Mobile phase model is related to formation of active forms of Cu, acting as leading molecules for Cu transport through the soil column. The migration of mobile ligands is quite fast, but at least some of them can be slowed and even blocked by the colloidal organic matter. This could be put in relation with the absence of low weight OM molecules like tyrosine, tryptophan like protein substances and sub-microbial protein products in the leachates. It is reasonable to suppose that the high dynamic of these DOM components ease the carrying of Cu downward, but they are also easily adsorbed by colloidal matter in soil column thus explaining the decrease of Cu leaching.

Proposed mechanisms

In accordance with these models, it was possible to propose some scenario explaining Cu fate in the case of different soils origin and different sludge characteristics (Figure 86).

In all grass soil and paddy soil, Cu will interact with immobile phase, colloids and mobile phase, but the level of interaction is different due to the type of sludge amendment and sort of soils. These interactions will affect to Cu leaching ability. So the change of symbol's size in Figure 86 represented the degree of implication of different phases in soil after sludge amendment. Besides, the size of symbol concerning sludge showed the Cu leaching ability in soil column, the size of orange symbol indicate the level of copper leaching efficiency.

The different proposed mechanisms are divided in four groups: **1) the control, 2) the group of composted (Cp1, Cp2), centrifuged (BC), reed bed (LR), centri-dried (BCD), 3) the group of limed (BCh), digested (BD), thickened (BE) and filter-pressed (BFP), and 4) the group of granular sludge.** The organic, mineral matter and clay contents in grass soil is higher than in paddy soil, explaining why the level of colloids and immobile phase in Figure 86d, e, f, g is higher than in Figure 86a, b, c, d.

The controls soil (Figure 86a and Figure 86e): Grass soil had higher organic matter and clay content compare to paddy soil. The colloidal contents in grass soil are therefore higher whatever the conditions. High DOC concentration was found in the leachates of grass soil and higher organic matter concentration in native grass soil, explaining why the size of symbol indicating immobile and mobile phase is higher in grass soil column (Figure 86e). The lower mobility of Cu in grass soil compare to paddy soil could be linked to higher colloids concentrations in grass soil, leading to slow phase interaction or more



immobilization of Cu and thus less mobile phase. Cu leaching is due to the Cu binding to colloids phases. The differences between Figure 86a and Figure 86e is the level of colloids. The high OM and clay content in grass soil lead to increase the content of colloids or slow mobile phase linking copper. This phenomena may reduce the copper release in grass soil compared to paddy soil.

The group of (Cp1, Cp2, BC, LR and BCD) (*Figure 86b and Figure 86f*): The application of composted, centri-dried or centrifuged sludge containing high immobile fraction of OM will lead to increase the colloidal concentrations for Cu adsorbed. Therefore, Cu stayed in soil column and was hardly leached out: all the symbol size are increasing due to higher DOC concentration in soil leachates (the orange symbol), higher organic matter (violet symbol) and larger mineral matter (green) of native sludge amendments compared to control soil (Figure 86a and Figure 86e). These figures demonstrate that the copper interaction with both slow and immobile phase in soil column explain why copper is retained in soil column. In parallel, the immobile phase provided by sludge in these columns increased copper leaching (comparing with control soil in Figure 86a and Figure 86e). However, the leaching of mobile phase reduces quickly according to the leaching process. That explains why low copper release percentages were found in the leachates of these sludges amended on soil column.

The group of (BCh BD BE and BFP) (*Figure 86c and Figure 86g*): On the other hand, limed sludge, filter-pressed had high proton/cation exchange capacity and DOC concentration which lead to increase the mobility of Cu in soil column and enhance the Cu desorption efficiency, especially when the size of soil particles was larger like in paddy soil. Besides, low OM in limed sludge amended soil provided negligible colloidal concentration for interaction with Cu ion in soil column. The size of green and violet symbol increase compare to control soil but were lower than Figure 86b and Figure 86f, the opposite trend was seen in orange symbol, due to the fact that BFP, BD, BE and BCh amendment contained lower organic and mineral matter, but higher DOC concentration than other conventional sludge. Evidently, due to the anaerobic degradation of stabilization process, digested sludge contain low molecular weight OM component. This mean that the digested sludge amended soil were containing a high number of mobile phase. As expected, digested amended soil column often presented high Cu leaching efficiency, especially in case of paddy soil. In Figure 86c and Figure 86g, the level of colloids increased compare to Figure 86a and Figure 86e but decreased compare to Figure 86b and Figure 86f respectively, due to sludge landspreading. These levels of colloids components were based on the organic matter contained in sludge amending. In limed, filter-pressed and digested sludge DOC was lower than in composted, red bed, centri-dried, centrifuged and reed bed sludge. The hypothesis is that the copper have more attractive interactions with the mobile phase for enhanced copper leaching ability. The difference between Figure 86c and Figure 86g is the level of slow mobile phase in Figure 86c. It could be explained by the large size of soil particular of paddy soil, which lead to reduce the gradient of colloidal between the top and bottom of soil column and led to remove the slow mobile phase in paddy soil column (Figure 86c).

The group of granular sludge (*Figure 86d and Figure 86h*): presented the highest level of colloid due to the increase organic matter content in native granular sludge amendment. Granular sludge amended



grass soil showed the highest DOC concentration in all five leachates which corresponded to the highest Cu leaching concentration in soil solution, compared to conventional sludge amended grass soil. This phenomenon is represented by an increase in the size of the orange symbol in Figure 86h and a lower size in Figure 86d. In case of paddy soil, the DOC concentration in leachates of granular sludge amended soil are generally lower than BCh, BFP, BD and BE amendment, thus explaining was the size of orange symbol was higher in Figure 86d, compared to Figure 86c. In the case of sludge BAN and BAE amended paddy soil, leaching percentage was slightly higher than some conventional sludge samples amendment such as composted (Cp1 and Cp2), thickened (BE), reed bed (LR) and centri-dried sludge (BCD) probably due to the increase of mobile phase after granular sludge amendment. In the case of sludge BAN and BAE amended paddy soil, leaching percentage was lower than limed (BCh), filter-press (BFP), digested (BD) and centrifuged (BC) sludge. It could be hypothesis that the complex organic complexes formed by these sludge samples were more mobile compare to those formed by granular sludge. Thus granular sludge landspreading limit in some case Cu desorption in paddy soils. The level of colloids observed in case of granular sludge amendment Figure 86d and Figure 86h, was high compared with other figures. The level of colloids in Figure 86h is higher than that in Figure 86d due to higher OM content in grass soil than in paddy soil. Figure 86d and Figure 86h explained the interaction of Cu with immobilized, mobile and slow mobile phase, which reflect the high copper interaction with soil component after granular sludge amendment leading to more copper leaching ability.

In conclusion, from the result obtained during this study we can highlight that the sludge amended soil leads to more copper interaction with sludge and soil component, and therefore change the copper leaching behavior. The different sludge samples affect copper mobilization due to the differences of their properties.

Granular sludge contained high material such as organic and mineral matter which increases the copper binding with colloids component in soil for better copper retention or copper exchange with other ion in slow or mobile phase for copper leaching. The similar phenomenon was also observed in the case of composted and dried and reed bed sludge and the opposite trend was seen in limed and digested sludge.

Besides, the results obtained in paddy and grass soil also demonstrated strongly the impact of human activities an environment condition to soil properties, and therefore copper availability after land spreading. In some case, like of granular sludge, composted and dried sludge, the application of sludge not only increase the nutrient content but also reduce to copper leaching ability.

Paddy soil from Vietnam contains low organic matter, the application of granular sludge as some other kind such as composted or dried sludge can not only increase the nutrient content but also reduce the copper leaching ability.



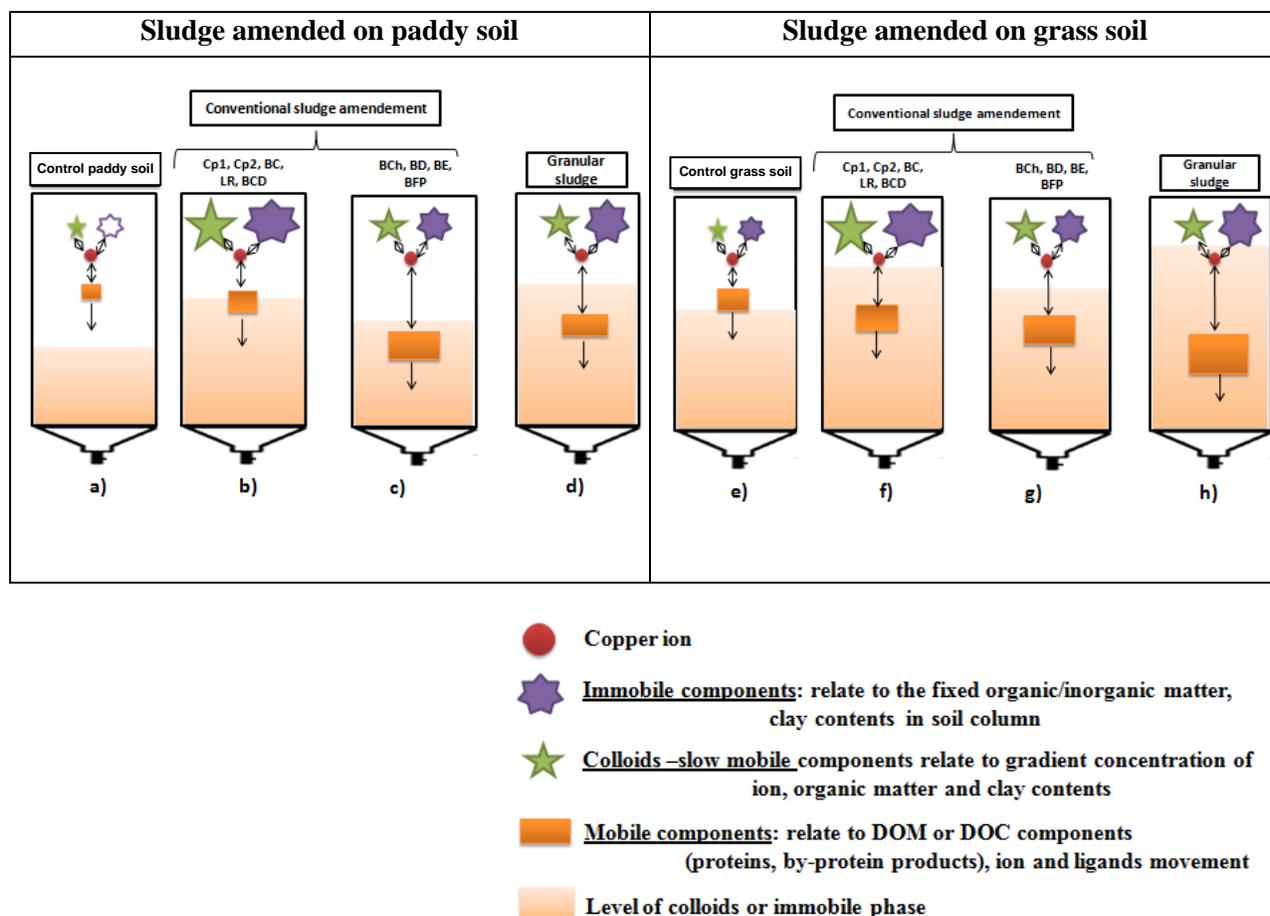


Figure 86: Proposed mechanism for Cu mobility in grass soil and paddy soil after sludge amendment

Prospect to broaden the study:

The methodology described in this work could be expanded and improve. Further choices need to be considered.

Using other types of soil, such as soil from other agricultural region in Vietnam (such as Delta or Mekong region) or in different French places, in order to have a better understanding of soil composition on leaching ability.

Application of the above described methodology to determine leaching ability of other contaminants such as As (of big interest in Vietnam), other trace metal element, or organic micropollutants.





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APPENDICES





APPENDICES

Appendices 1: Granules production

1. Introduction

Optimization of water treatment processes requires the integration of new parameters for the system approach such as improving productivity and reliability, minimizing sludge production and oxygen consumption, the economy or the recovery of energy and matter, the increase in the efficiency of the oxidation reactions and the solid-liquid separation.

Wastewater treatment technologies such as conventional activated sludge is confronted with disadvantages like production of high residue biomass, high concentration of suspended solids in output, large area for construction, low processor load (Corbitt, 1999; Metcalf and Eddy, 2003), ... Moreover, settling possibility of the traditional activated sludge is quite low, which increases sludge disposal and cost of construction.

Thus, new methods may become useful to overcome these various drawbacks. The use of granular sludge in a sequenced aerobic fluidized system is one of the proposals for improvement of the treatment processes.

Aerobic granules system shows advantages compared to the conventional activated sludge, such as good settling ability, maintaining high biomass concentration, suitable with high organic substances, dense and solid structure and able to treat organic matter and nitrogen simultaneously.

Nevertheless, there are many factors influencing formation and properties of granules: hydraulic, settling time, dissolved oxygen, hydraulic retention time, substrates, temperature....

In this study, we wanted to use the structural features of the granules to compare their sorption properties with the other matrices tested. For this we have implemented processing pilots whose operation is described in this chapter and try to optimize the settling time.

2. Pilots for granules production

Two identical sequential air-lift bioreactors (50 cm in height and 5 cm in diameter with a working volume of 1 L) were used for granular formation (figure 1). Seed sludge was taken from an aeration tank of the sewage treatment plant of Limoges (France) which is a 240 000 Inhabitant equivalent. The seeding sludge had a fluffy, irregular and loose-structure, and was brown in color.

Aeration airflow rate was around 1.2 L/min, equivalent to a superficial up-flow air velocity of 0.6 cm/s.



A 4-h operation cycle was implemented in each reactor operation. In every cycle half of the reactor volume was exchanged by feeding (the hydraulic retention time (HRT) is 8 hours). The operating cycle of R1 was 2-min feeding, 15 min of anoxic period with mechanical mixing, 198 min of aeration with 15 first minutes of mixing (air and mechanical mixing), 4 to 2 min settling, 2-min withdraw and 10-min idling. After 5 days, 2 minutes of settling time was imposed. For reactor R2, the pattern of operation with feeding, anoxic, and withdraw was identical to that of R1 excepted for settling which was gradually decreased: 0-16th day: 15 min, 17-22nd day: 5 min, 22-25th day: 4 min, and 3 min of settling time was kept until the end of experiment.

A synthetic wastewater with the following compositions was used as influent for cultivation of aerobic granules in the reactors: COD (sodium acetate): 1300 mg l⁻¹, NH₄Cl: 248 mg l⁻¹, NaNO₃: 400 mg l⁻¹, K₂HPO₄: 73 mg l⁻¹, CaCl₂·2H₂O: 30 mg l⁻¹, MgSO₄·7H₂O: 25 mg l⁻¹, FeSO₄·7H₂O: 20 mg l⁻¹, H₃BO₃: 0.05 mg l⁻¹, ZnCl₂: 0.05 mg l⁻¹, CuCl₂: 0.03 mg l⁻¹, MnSO₄·H₂O: 0.05 mg l⁻¹, (NH₄)₆Mo₇O₂₄·4H₂O: 0.05 mg l⁻¹, AlCl₃: 0.05 mg l⁻¹, CoCl₂·6H₂O: 0.05 mg l⁻¹, and NiCl₂: 0.05 mg l⁻¹. The concentrations of other components were same as those reported by Liu and Tay (2007). The pH of feed solution was kept between 6.3 and 7.

All analyses are describes in the Material and methods part.

2.1. Substrate removal

Organic substrate was treated effectively for both reactors (Figure 87). For first 10 days, COD removal rate of the reactor increased firstly in both reactors R1 and R2. After that, the organic removal was very effective. CODs in the effluent of R2 were below 50 mg O₂/L while in R1, from day 15 to day 21, COD in the effluent was increase because a wash out of the biomass

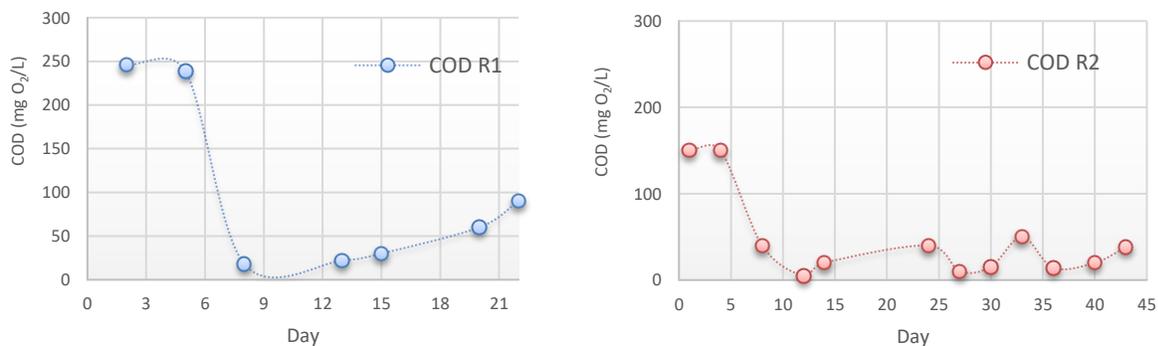


Figure 87 : Substrate removal of R1 and R2

The degradation profile of organic substrate of R1 and R2 during the oxidation phase was showed in Figure 88. COD decrease closely to minimum after 90 minutes for both reactor R1 and R2. The concentration of dissolved oxygen in the reactor showed that in the first 50 min, bacteria uptake strongly oxygen for organic substrate, so DO in the reactor experienced a very gradually increase.

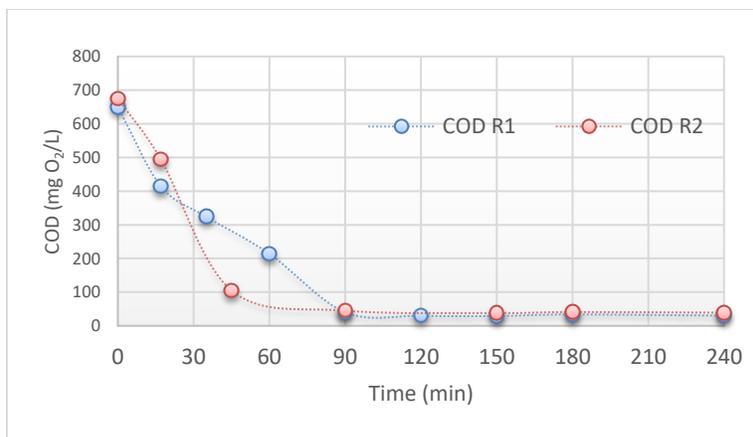


Figure 88. Substrate degradation in one cycle

2.2. Monitoring the granulation: biomass characteristic and settling properties

2.2.1. Settleability

In R1, during the entire of 21-days operation, SVI value never below 200 ml/g. From 1st to 8th day, SVI decrease from 322 to 200 ml/g (Figure 89). Note that after day 5, settling time was adjusted to 2 min, the significant increase in SVI was observed when granular sludge was in formation. At day 19, the SVI value was 334 ml/g, meaning that the settleability was worse than initial. The out-growth of filamentous bacteria in or on aerobic granule causes poor settleability and subsequently the washout of biomass, as indicated by a drop in biomass concentration. Filament appeared on the surface of granular led to decline the density, increase porosity and thus reducing the settleability (Figure 89).

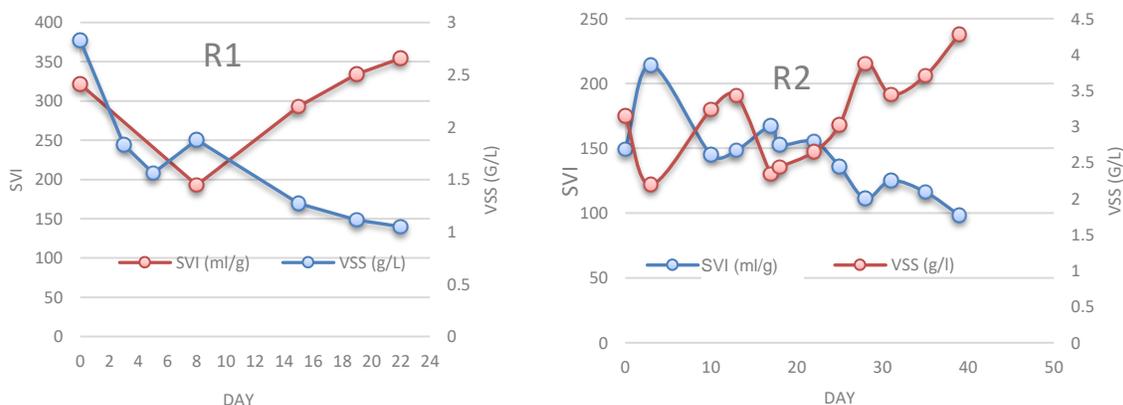


Figure 89: Biomass characteristic and settling properties of R1

Overall profiles of granules SVI of R2 (Figure 89– R2) increased in the first 3 days which indicated that the settling properties of aerobic granular sludge were getting poor during this period. The hypothesis was



because aerobic granules required a process to adapt to the new environment. Then, overall profiles of SVI were a downward trend during the 3rd to the 39th day which both reached the minimum of 98 ml/g on the 39th day. In this period, there were some fluctuation of SVI on day 15 and 23, attributed to the wash out of biomass when starting decrease settling time.

Thus, granular sludge could have good settling performance under selection pressure according to the result obtained.

As apparent on Figure 4, in the first 3 day operating, the VSS of reactor R2 decreased from 3.15 to 2.2, followed by the increase of SVI index (from 150 to 214). Because fresh sludge was taken from wastewater treatment plant of Limoges and used directly as inoculums to the SBR, it needed some days of adaptation with the new environment. From day 3 to day 13, VSS in the reactor went up quickly (Figure 89), reached 3.43 g/L VSS at day 13. After 16 day operating with a settling time 15 min, the settling time was set up to reduce to 5 minutes to gradually increase a selection stress.

After declining sedimentation time to 5 minutes, a major washout occurred right after decreasing the settling time, which resulted in a sharp decrease of biomass content of the system from 3.43 to 2.34g VSS/L. Yet, VSS values recovered rapidly and biomass concentrations increased significantly in the following days. After decreasing the settling time to 4 min and 3 min at day 23 and 26, biomass concentration did not be washed out, but also surge up dramatically. The biomass had some fluctuation at day 28 but the trend was increase. At day 39, VSS value was 4.29 g/L. The high biomass concentration while exceptionally good settleability (SVI=98 ml/g) of the granular sludge confirmed that stepwise reduce settling time is very good strategy for cultivation aerobic granular sludge. Gradually reducing settling time are applied and the system is given sufficient time to adapt to new conditions, it was possible to maintain considerable amount of biomass with remarkably good settling properties, despite stress conditions (Sadrzadeh and Dulekgurgen 2014).

Comparing two reactor R1 and R2, the granulation rate of R1 was much faster than R2. With the same organic loading rate, the F/M ratio depended on the quantity of biomass in the reactor. Reactor 1 had a settling time of 2 min after 5 days, caused a wash out of large quantity of biomass. In contrary, in the reactor R2 was applied stepwise settling time always maintaining biomass in the reactor (most of the time VSS>3g/L). Thus, F/M, or biomass loading rate of R1 was always much higher than R2. It was reported that F/M ratio displayed a significant effect on the rate of granulation and the morphology property of the granules (Li et al. 2011). With the same loading rate of biomass, the lower biomass in the reactor can form faster formation and larger granules (Li et al. 2011).

2.2.2. Morphology of granular sludge

The seeding sludge was typical activated sludge with irregular, dispersed in the mix liquor with brown color. At day 6, the sludge was still full of filament, and no evidence of granular formation (Figure 90a). However, the color of sludge changed from dark brown to yellow-brown. At day 11, the initial formation of small aerobic granules could be seen with many flocs sludge aggregate together (Figure 90b). After 14 days, many



granules were formed with diameter range from 300 micrometers to 1 mm and filamentous structures could be observed at the surface of the granules. At day 21, the SBR was dominated by the granules with size 1-3 mm.

The granules were divided into two parts: the filamentous part had white color with full of filaments bacteria, while inner part had yellow-brown color (Figure 90d). It was fluffy and not stable. Under the shear force, the outer part of the granular began to disintegrate afterwards leading the significantly increase of flocculent sludge in the mix liquor.

In case of R2, because of the gradually decrease settling time; allow keeping high concentration of biomass in the reactor. The growth of sludge was much slower than those in R1. After 7 days, the morphology of sludge was quite the same with the initial: all sludge was floc-like and dispersed (Figure 91a). At day 14, it was clear that the granulation was happened with the appearance of many small granules. Nevertheless, suspense sludge was still dominated in these days comparing with granular. The granulation process was developing with the increase of the size and the quantity of granule. After 21 days cultivating in the reactor, granular sludge with size around 500 μm to 1 mm, which has yellow- brown color and clear spherical shape was dominant in the reactor. Filamentous bacteria completely disappeared in the reactor (Figure 91c). At days 42, aerobic granules increased in size and more compact. The mean diameter was 1-1.3 mm (Figure 91e)

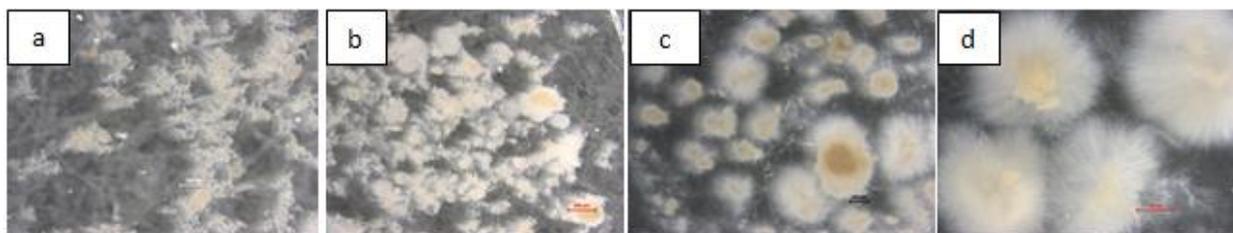


Figure 90: Image analysis photographs of bioflocs/granules of R1 on day [a] 6th day, [b] 11th day, [c] 14th day, [d] 21st day. (bar=500 μm)



Figure 91: Image analysis photographs of bioflocs/granules of R1 on day [a] 7th day, [b] 14th day, [c] 21th day, [d] 28st day, [e] 42nd day (bar= 500 μm)

The detailed microstructure of the granular sludge of R1 and R2 was examined using scanning electron microscope (SEM).

Microbial distribution of granular taken in reactor R1 was shown in Figure92. The surface of the granule was uneven, porous and weak structure, and there existed a large number of channels. The outer part of



granular was cover by filaments; while in the interior of granules filamentous and bacillus (rod-shape) bacteria coexisted. However, filamentous were more dominant comparison with rod-shape bacteria.

Sludge taken in day 28 in reactor R2 was observed by SEM (Figure 93). The surface of the granule was uneven but much more smooth than granular in reactor R1. The granules had rich biological species inside, which included bacillus, coccus and even protozoa (ciliate). The co-existence of bacillus and cocci and adhered together filaments were observed in the surface of the granules in the pilot R2. Since the growth of nitrifying bacteria was much slower than heterotrophs (Sharma and Ahlert, 1977), aerobic granules may offer a protective matrix for nitrifying population to grow without the risk of being washed out from the system and protecting the anaerobic core inside the granule (Liu et al. 2004). In addition, the nitrifying populations have highly hydrophobic interactions, it is concluded that their growth could contribute to the higher cell hydrophobicity and facilitate the cell-cell interaction (Kim et al. 2000). EPS cover all the surface of granular and the dominant of bacillus, coccus in the surface made granular in R2 was very strong structure, dense and compact.

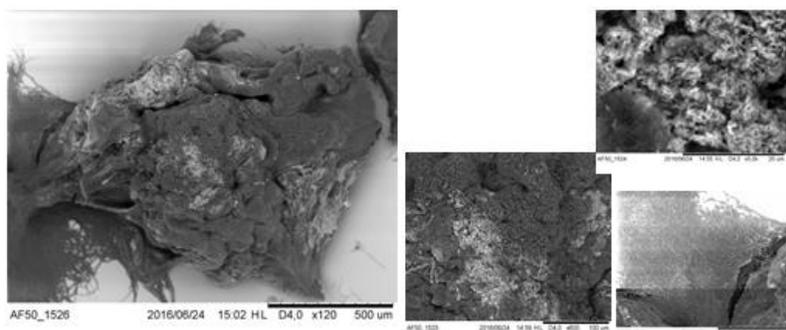


Figure92: SEM image of granular sludge taken in R1 day 17

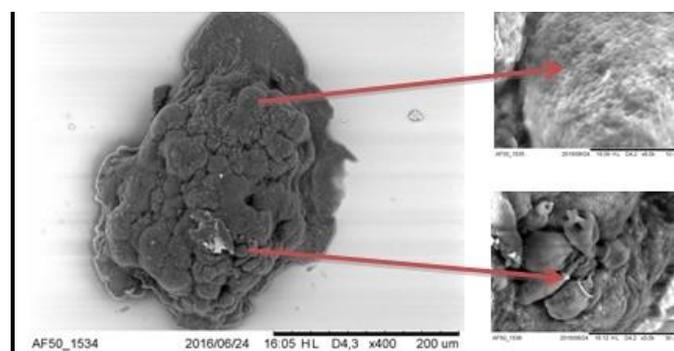


Figure 93: SEM image of granular sludge taken in R2 day 28

2.2.3. Sludge EPS during the granulation

Variation of extra polymeric substance of sludge in reactor R1 and R2 was illustrated in Figure 94. Figure 94 showed the change of EPS concentration and the ratio of PN/PS during the granulation of R1



and R2. It was observed that the amount of protein was much higher than those of polysaccharide in component of EPS sludge in both two reactors. Nevertheless, for 2 reactor operations with the different mode of settling time, even the protein was the main component, the PN/PS ratio in R1 decrease with increase the operation time.

If COD was sufficient for biomass growth, the excess carbon sources was transferred either as intracellular substances for storage or for extracellular biopolymers. These extracellular biopolymers might be used for enrich of the polysaccharide contents in EPS (Zhang et al. 2007). In term of total EPS, after the formation of granular (day 8th) until the failure of the system, the total EPS was down trend significantly. At day 20, the EPS which eventually fell to about 5.8 mg/g MLVSS at the end of the experiment and PN/PS ratio was dropped to 5 (note that initial PN/PS ratio was 20). The downtrend of EPS came from the disintegration of granular sludge.

The trend variation of PN/PS and total EPS of reactor R2 was at the opposite (Figure. 9 R2). The PN/PS ratio and total EPS was going up gradually during the operation time. At day 45th, PN/PS was 27 and total EPS after extracting from sludge was 76 mg/g VSS. With the increase of PN/PS, it can be concluded that the PN play an important role for granulation as written in literature part. This result was consistent with the previous findings for the EPS composition in aerobic granules (Li et al. 2011). Proteins have been reported as the core EPS constitute of the aerobic granules, which were believed to be the important building materials for the internal structure of granule (Li et al. 2011).

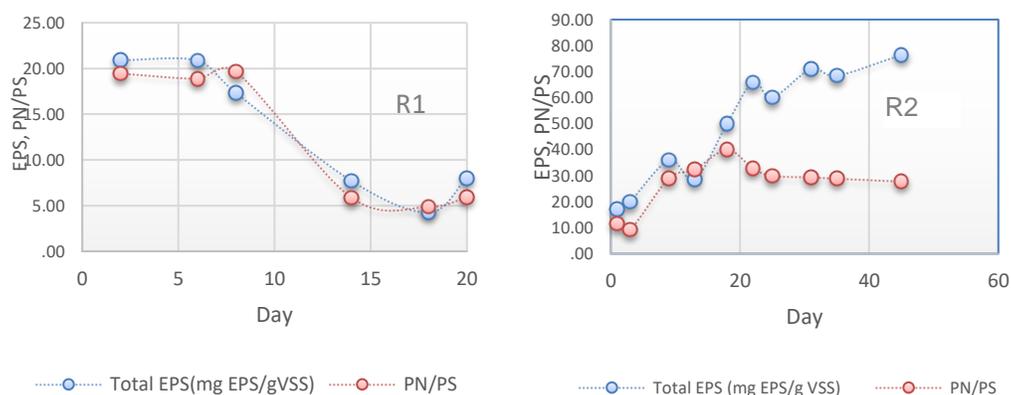


Figure 94: Variation of EPS during granulation of R1 and R2

Table 35 briefly summarized the change of EPS for 45 days of operation for R2 reactor. Initially, lipid presented as 2.6 mg/g VSS in sludge but after 45 days, it contributed 5.2 mg/g VSS. The lipid in granulation after 45 days was double than in the initial sludge. The high amount of lipid in the in granule presented the hydrophobicity of sludge. It has been recognized that the hydrophobicity of the cell surface plays an important role in the self-immobilization and attachment of cells to a surface and thus trigger the granulation (Tay et al. 2001).

Table 35 : EPS in R2-granules

Day	0	45
Protein (mg/g VSS)	18.8	201.8
Humic acid (mg/g VSS)	15.0	32.4
Polysaccharide (mg/g VSS)	1.6	7.2
Lipid (mg/ g VSS)	2.6	5.2

Figure 96 and Figure 97 showed the distribution of protein and polysaccharide of the granular sludge in the reactor R1 and R2. For R1, structure of granular with full of filamentous fungi in the surface and the core of the granule was not observed relevantly. Constantly, granular sludge in R2 reactor showed clearly the layer structure. For both of the granular R1 and R2, the florescence intensity of protein (by FITC-green color) was much higher than those for polysaccharide (ConA-red color), explained that protein content was much higher than polysaccharide. It is similar to the results analyzing with biochemical method and relating to report of McSwain et al (2005), which protein was the main component constituting to EPS. However, report showed that the center of the granules was mostly stained by FITC, which stains cells or free amino groups, and polysaccharides were at the edge of the section (Chen et al. 2007). It was contrary to our results. Regarding to the distribution of protein and polysaccharide of granule in R2, both protein and polysaccharide were distributed in the outer part of granular, and the signal at the core was very week.

From the bibliography, as the particle size increases, so does the mass transfer limitation of oxygen within the outer layer of active biomass? Mass transport limitations eventually create various layers of aerobic, anaerobic, and dead biomass within granules. The aerobic layer of biomass has been reported to be 800 μm in diameter (McSwain et al. 2005), which is much longer than observed in the present study. The exact structure of aerobic granules is probably dependent on reactor operation, species selection, and biofilm growth morphology (McSwain et al. 2005). It is supposed that the inner core of granule was precipitate of inorganic component like carbonate, phosphate of calcium or magnesium. It was reported that calcium concentration in anoxic/aerobic reactor during the anoxic phase when pH reached nearly 9, tends to show the calcium phosphate precipitation with the form $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ together small amount of $\text{Ca}_{18}\text{Mg}_2\text{H}_2(\text{PO}_4)_{14}$ (Filali et al, 2012. Juang et al, 2010). These precipitations deposit in the inner part of the aerobic granules enhance the granule stability (Mañas et al, 2011). Additionally, Ca^{2+} can bound with extracellular polymeric substances (EPS), and further helped to strengthen the structure and favor the formation of big and fast-settling of granules (Liu et al, 2011, Caudan et al, 2012. Caudan et al, 2014). It needs to have some experiment like X-ray diffraction (XRD) or Fourier transform infrared spectroscopy (FT-IR) to confirm this hypothesis.



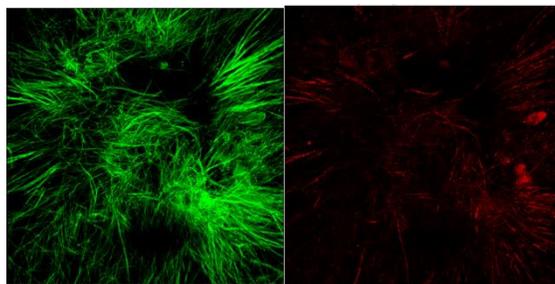


Figure 95: Distribution of protein (green) and polysaccharide (red) of granular in R1

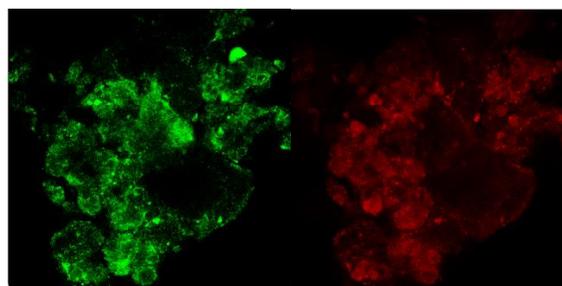


Figure 96: Distribution of protein (green) and polysaccharide (red) of granular in R2

The size of granular of R1 was 2-3 mm and 0.5-1 mm for R2. As it was mentioned from previous, granular sludge from R1, in spite of having large size, was fluffy and weak structure, full filamentous in the surface and having more problems in long-term operation. Comparison with granular in R2, it was very dense and compact, as can be seen in Figure 93.

Since all the other operational conditions were same except for the strategies of settling time, it was believed that the difference of granulation morphology was due to the operational mode of settling time, which influenced the granulation significantly.

3. Conclusions

The settling time acts as a major hydraulic selection pressure on microbial community (Wang et al. 2011). In SBR operation, only particles which could settle in given time frame could stay in the reactor; while poor settling particles were wash out the cycle. Hence, settling was fundamental factor for trigger sludge formation and it was also considered as physical screening step to select the good settling bacteria in the reactor.

Comparing two reactor R1 and R2, the granulation rate of R1 was much faster than R2. With the same organic loading rate, the F/M depended on the quantity of biomass in the reactor. Reactor R1 having a settling time of 2 min after 5 days, caused a wash out of large quantity of biomass. In contrary, in the reactor R2 was applied stepwise settling time always maintaining biomass in the reactor (most of the time VSS > 3g/L). Thus, F/M, or biomass loading rate of R1 was always much higher than R2. It was reported that F/M ratio displayed a significant effect on the rate of granulation and the morphology property of the granules (Li et al. 2011).



With the same loading rate of biomass, the lower biomass in the reactor can form faster formation and larger granules (Li et al. 2011). The size of obtained granular of R1 was 2-3 mm and 1-1.3 mm for R2. As it was mentioned from previous, granular sludge from R1, even has large size, was fluffy and with a weak structure, full of filament in the surface and have more problems in long-term of operation. Comparison with granules in R2, it was very dense and compact. Liu and Tay (2015) also compare the sludge growth rate of sludge with different strategies of settling. They found that the growth rate of sludge when applied fixed settling time at 2 min was 3.0-fold higher than that with stepwise settling mode.

Since all the other operational conditions were same except for the strategies of settling time, it was believed that the difference of granulation morphology was due to the operational mode of settling time, which influenced the granulation significantly.

In conclusion, the strategy fixed settling time was performed with very fast formation of the aerobic granules. However, the granules were formed with filamentous structure, high porosity, instability and results in the not feasibility for practical application. Applying gradually decrease of settling time strategy could form small granules but compact, dense with no outgrowth of filament on the surface and stable for more than 40 days without collapse the system. It was proved to be suitable for removing slowly settling flocks and retaining high amounts of biomass with improved settling properties: substantial biomass washout occurred each time settling time was reduced, but biomass recovery and stable biomass with significantly improved settling properties.

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Appendices 2 : Complements to the results and discussion of biochemical components and functional groups of sludge (chapter 1)

Table 36: Identification of fluorescence peak for spectra of soluble fraction of different sludge

Sludge	Zone I (trypsin proteins-like)		Zone II (tryptophane proteins-like)		Zone III (microbial proteic-like by-products)		Zone IV (fulvic Acid-like)		Zone V (Humic acid-like)	
	Ex/Em wavelengths (nm)	Relative intensity	Ex/Em wavelengths (nm)	Relative intensity	Ex/Em wavelengths (nm)	Relative intensity	Ex/Em wavelengths (nm)	Relative intensity	Ex/Em wavelengths (nm)	Relative intensity
BE (WWTP1)	275/303	269	265/353	183	325/368	31	325/430	181	255/416	31
BCh (WWTP1)	275/304	226	275/343	142	330/374	38	-	-	-	-
BD (WWTP1)	280/310	304	275/333	140	330/350	129	320/436	165	250/445	356
BCD (WWTP1)	275/303	180	275/341	109	330/375	51	330/410	47	250/419	51
BFP (WWTP1)	270/298	713	275/332	307	330/375	17	-	-	-	-
BC (WWTP2)	270/298	411	275/348	88	315/355	113	-	-	-	-
Cp1 (WWTP2)	280/311	137	-	-	330/376	235	330/422	306	250/443	360
Cp2 (WWTP2)	275/304	35	-	-	335/382	61	325/422	84	250/435	94
LR (WWTP3)	275/304	404	275/329	237	330/375	45	335/431	57	474.978	106
BAN	275/305	496	275/346	278	315/376	84	325/430	62	255/430	37
BAE	275/307	389	275/339	215	330/375	79	335/430	32	250/420	16



Appendices 3: Complements to the results and discussion for copper leaching ability in leachates and leaching solution characteristics after landspreading on soil column experiments (chapter 3)

Table 37 : Cu leaching percentages in leachates collected after each two days of leaching process compared to total copper leached from sludge amended grass soil column

Sludge amended grass soil		Cu leaching percentages in grass soil leachates (%)				
		L1	L2	L3	L4	L5
Control soil		50.76 ± 5.76	14.02 ± 4.02	24.15 ± 4.15	8.23 ± 2.23	2.83 ± 0.83
Conventional activated sludge	BE (WWTP1)	34.74 ± 4.47	11.08 ± 1.08	34.74 ± 4.74	10.67 ± 1.67	8.76 ± 1.76
	BCh (WWTP1)	27.19 ± 7.19	3.68 ± 0.68	15.44 ± 5.44	29.47 ± 2.47	24.22 ± 2.22
	BD (WWTP1)	34.30 ± 4.30	17.50 ± 1.50	12.97 ± 2.97	19.05 ± 1.05	16.18 ± 1.18
	BFP (WWTP1)	29.34 ± 2.34	17.06 ± 1.06	10.43 ± 1.43	29.68 ± 2.68	13.49 ± 3.49
	BCD (WWTP1)	53.69 ± 3.69	11.02 ± 1.02	11.28 ± 1.28	13.32 ± 3.32	10.69 ± 1.69
	BC (WWTP2)	57.37 ± 7.37	13.26 ± 3.26	11.41 ± 1.41	9.89 ± 0.89	8.08 ± 0.08
	Cp1 (WWTP2)	47.87 ± 7.87	14.11 ± 4.11	14.46 ± 4.46	11.73 ± 1.73	11.84 ± 1.84
	Cp2 (WWTP2)	30.22 ± 3.22	14.63 ± 1.63	19.21 ± 9.21	21.68 ± 1.68	14.26 ± 4.26
LR (WWTP3)	34.50 ± 4.50	14.22 ± 1.22	24.22 ± 2.22	15.16 ± 1.16	11.90 ± 1.90	
Granular sludge	BAN	23.37 ± 3.37	19.60 ± 1.60	22.58 ± 2.58	25.42 ± 2.42	9.02 ± 1.02
	BAE	20.27 ± 2.27	18.10 ± 1.18	30.96 ± 3.96	20.90 ± 2.90	9.77 ± 1.77



Table 38 : Cu leaching percentages in leachates collected after each two days of leaching process compared to total copper leached from sludge amended paddy soil column

Sludge amended paddy soil		Cu leaching percentages in paddy soil leachates (%)				
		L1	L2	L3	L4	L5
Control soil		77.94 ± 7.94	17.55 ± 7.55	4.51 ± 1.51	ND	ND
Conventional activated sludge	BE (WWTP1)	44.81 ± 4.81	15.80 ± 5.80	39.39 ± 9.39	ND	ND
	BCh (WWTP1)	47.86 ± 7.86	33.03 ± 3.03	16.46 ± 6.46	2.65 ± 0.65	ND
	BD (WWTP1)	45.5 ± 5.50	28.58 ± 8.58	18.38 ± 8.38	4.43 ± 0.43	3.11 ± 0.11
	BFP (WWTP1)	41.2 ± 1.20	43.46 ± 3.46	10.44 ± 1.44	3.65 ± 0.65	1.24 ± 0.24
	BCD (WWTP1)	68.42 ± 8.42	14.86 ± 4.86	9.52 ± 1.52	7.20 ± 1.20	ND
	BC (WWTP2)	21.41 ± 1.41	29.49 ± 2.49	32.46 ± 2.46	8.74 ± 1.74	7.9 ± 0.90
	Cp1 (WWTP2)	8.54 ± 0.50	13.90 ± 3.90	ND	ND	ND
	Cp2 (WWTP2)	24.16 ± 4.16	7.23 ± 1.23	68.61 ± 8.61	ND	ND
	LR (WWTP3)	19.43 ± 9.43	40.24 ± 4.24	40.33 ± 1.33	ND	ND
Granular sludge	BAN	49.00 ± 4.00	31.63 ± 3.63	13.33 ± 3.30	6.07 ± 1.07	ND
	BAE	52.80 ± 2.80	39.32 ± 8.23	8.88 ± 1.88	ND	ND

ND: none-detection

Table 39 : RP values of leachates collected after each two days of leaching process from sludge amended grass soil columns

Sludge amended grass soil		RP value in grass soil leachates (mV)				
		L1	L2	L3	L4	L5
Control soil		207.7 ± 17.7	248 ± 14.8	39.0 ± 3.9	101.2 ± 15.2	35.3 ± 5.3
Conventional activated sludge	BE (WWTP1)	192 ± 12.9	138.4 ± 13.8	147.1 ± 14.7	149.1 ± 14.9	135.5 ± 13.5
	BCh (WWTP1)	310 ± 13.1	181.7 ± 18.1	182.5 ± 18.2	175.8 ± 17.5	179.0 ± 17.9
	BD (WWTP1)	232.3 ± 12.3	47.4 ± 17.4	115.4 ± 15.4	128.7 ± 12.8	128.9 ± 12.8
	BFP (WWTP1)	263.7 ± 12.6	169.1 ± 16.9	165.1 ± 16.5	164.1 ± 16.4	161.5 ± 16.1
	BCD (WWTP1)	240 ± 12.4	122.0 ± 12.2	148.2 ± 14.8	165.2 ± 16.5	146.2 ± 14.6
	BC (WWTP2)	210.8 ± 12.2	169.5 ± 16.9	158.1 ± 15.8	144.6 ± 14.4	125.9 ± 12.5
	Cp1 (WWTP2)	239.4 ± 18.4	173.8 ± 13.8	174.4 ± 14.4	169.3 ± 19.3	81.9 ± 8.9
	Cp2 (WWTP2)	196.6 ± 16.6	145.3 ± 15.3	142.7 ± 12.7	127.2 ± 12.7	121.0 ± 12.1
	LR (WWTP3)	204.1 ± 12.0	157.4 ± 15.7	131.5 ± 12.1	104.8 ± 10.4	144.1 ± 14.4
Granular sludge	BAN	240.2 ± 12.2	188.5 ± 13.8	189.5 ± 12.9	151.6 ± 11.6	168.1 ± 15.8
	BAE	252.5 ± 12.5	170.0 ± 11.7	180.0 ± 11.8	188.0 ± 18.8	111.5 ± 11.1



Table 40 : RP values of leachates collected after each two days of leaching process from sludge amended paddy soil columns

Sludge amended paddy soil		RP value in paddy soil leachates (mV)				
		L1	L2	L3	L4	L5
Control soil		329.8 ± 17.7	243.1 ± 14.8	148.2 ± 13.9	211.6 ± 15.2	140.3 ± 15.3
Conventional activated sludge	BE (WWTP1)	303.2 ± 12.9	237.6 ± 13.8	123.3 ± 14.7	186.4 ± 14.9	108.7 ± 13.5
	BCh (WWTP1)	320.5 ± 13.1	257.1 ± 18.1	236.0 ± 18.2	214.3 ± 17.5	176.0 ± 17.9
	BD (WWTP1)	326.2 ± 12.3	270.9 ± 17.4	237.8 ± 15.4	239.7 ± 12.8	196.4 ± 12.8
	BFP (WWTP1)	315.7 ± 12.6	251.8 ± 16.9	247.9 ± 16.5	235.2 ± 16.4	174.6 ± 16.1
	BCD (WWTP1)	348.8 ± 12.4	254.9 ± 12.2	255.8 ± 14.8	233.5 ± 16.5	206.5 ± 14.6
	BC (WWTP2)	241.4 ± 12.2	136.1 ± 16.9	160.5 ± 15.8	140.1 ± 14.4	145.6 ± 12.5
	Cp1 (WWTP2)	347.8 ± 18.4	252.4 ± 13.8	244.4 ± 14.4	237.6 ± 19.3	211.4 ± 8.9
	Cp2 (WWTP2)	302.2 ± 16.6	243.9 ± 15.3	182.5 ± 12.7	210.1 ± 12.7	122.8 ± 12.1
LR (WWTP3)	359.2 ± 12.0	242.8 ± 15.7	134.0 ± 12.1	181.9 ± 10.4	124.0 ± 14.4	
Granular sludge	BAN	332.3 ± 12.2	251.3 ± 13.8	222.9 ± 12.9	208.0 ± 11.6	180.9 ± 15.8
	BAE	265.3 ± 12.5	231.0 ± 11.7	160.1 ± 11.8	174.8 ± 18.8	185.5 ± 11.1

Table 41 : UV absorbance at 254 nm of leachates collected after each two days of leaching process from sludge amended grass soil columns

Sludge amended grass soil		UV absorbance of grass soil leachates (abs)				
		L1	L2	L3	L4	L5
Control soil		1.09 ± 0.09	0.80 ± 0.06	0.89 ± 0.07	0.94 ± 0.08	0.96 ± 0.08
Conventional activated sludge	BE (WWTP1)	1.34 ± 0.11	1.13 ± 0.09	1.29 ± 0.10	1.04 ± 0.08	1.16 ± 0.09
	BCh (WWTP1)	2.42 ± 0.19	1.95 ± 0.16	1.17 ± 0.09	1.58 ± 0.13	2.05 ± 0.16
	BD (WWTP1)	2.21 ± 0.18	1.90 ± 0.15	1.28 ± 0.10	1.70 ± 0.14	2.31 ± 0.18
	BFP (WWTP1)	2.32 ± 0.19	1.40 ± 0.11	1.02 ± 0.08	1.43 ± 0.11	2.23 ± 0.18
	BCD (WWTP1)	2.17 ± 0.17	1.52 ± 0.12	1.12 ± 0.09	1.47 ± 0.12	2.17 ± 0.17
	BC (WWTP2)	2.16 ± 0.17	1.53 ± 0.12	0.86 ± 0.07	1.11 ± 0.09	1.26 ± 0.10
	Cp1 (WWTP2)	1.67 ± 0.13	0.79 ± 0.06	0.68 ± 0.05	0.78 ± 0.06	1.63 ± 0.13
	Cp2 (WWTP2)	1.52 ± 0.12	1.09 ± 0.09	0.06 ± 0.05	1.22 ± 0.10	1.36 ± 0.11
LR (WWTP3)	1.69 ± 0.14	1.19 ± 0.10	1.02 ± 0.01	1.21 ± 0.10	1.46 ± 0.12	
Granular sludge	BAN	2.74 ± 0.22	2.66 ± 0.21	1.38 ± 0.08	1.15 ± 0.09	1.29 ± 0.10
	BAE	2.84 ± 0.23	2.36 ± 0.19	1.85 ± 0.11	1.36 ± 0.11	1.17 ± 0.09



Table 42 : UV absorbance at 254 nm of leachates collected after each two days of leaching process from sludge amended paddy soil columns

Sludge amended paddy soil		UV absorbance of paddy soil leachates (abs)				
		L1	L2	L3	L4	L5
Control soil		0.49 ± 0.04	0.29 ± 0.04	0.30 ± 0.07	0.34 ± 0.06	0.25 ± 0.05
Conventional activated sludge	BE (WWTP1)	1.85 ± 0.17	0.47 ± 0.04	0.67 ± 0.06	0.63 ± 0.06	0.55 ± 0.05
	BCh (WWTP1)	0.82 ± 0.07	0.33 ± 0.03	0.32 ± 0.03	0.48 ± 0.04	0.45 ± 0.04
	BD (WWTP1)	0.73 ± 0.07	0.41 ± 0.04	0.48 ± 0.04	0.42 ± 0.04	0.74 ± 0.07
	BFP (WWTP1)	1.35 ± 0.12	0.44 ± 0.04	0.41 ± 0.04	0.48 ± 0.04	0.65 ± 0.06
	BCD (WWTP1)	0.68 ± 0.06	0.39 ± 0.03	0.41 ± 0.04	0.52 ± 0.04	0.39 ± 0.03
	BC (WWTP2)	1.21 ± 0.11	0.55 ± 0.05	0.43 ± 0.04	0.43 ± 0.05	0.37 ± 0.03
	Cp1 (WWTP2)	0.57 ± 0.05	0.38 ± 0.03	0.36 ± 0.03	0.38 ± 0.04	0.55 ± 0.03
	Cp2 (WWTP2)	0.81 ± 0.07	0.65 ± 0.06	0.78 ± 0.07	0.92 ± 0.03	1.02 ± 0.05
	LR (WWTP3)	0.65 ± 0.06	0.34 ± 0.03	0.48 ± 0.04	0.55 ± 0.04	0.46 ± 0.09
Granular sludge	BAN	1.25 ± 0.11	0.74 ± 0.07	0.42 ± 0.04	0.38 ± 0.04	0.32 ± 0.04
	BAE	0.84 ± 0.08	0.97 ± 0.09	0.68 ± 0.06	0.32 ± 0.06	0.24 ± 0.02



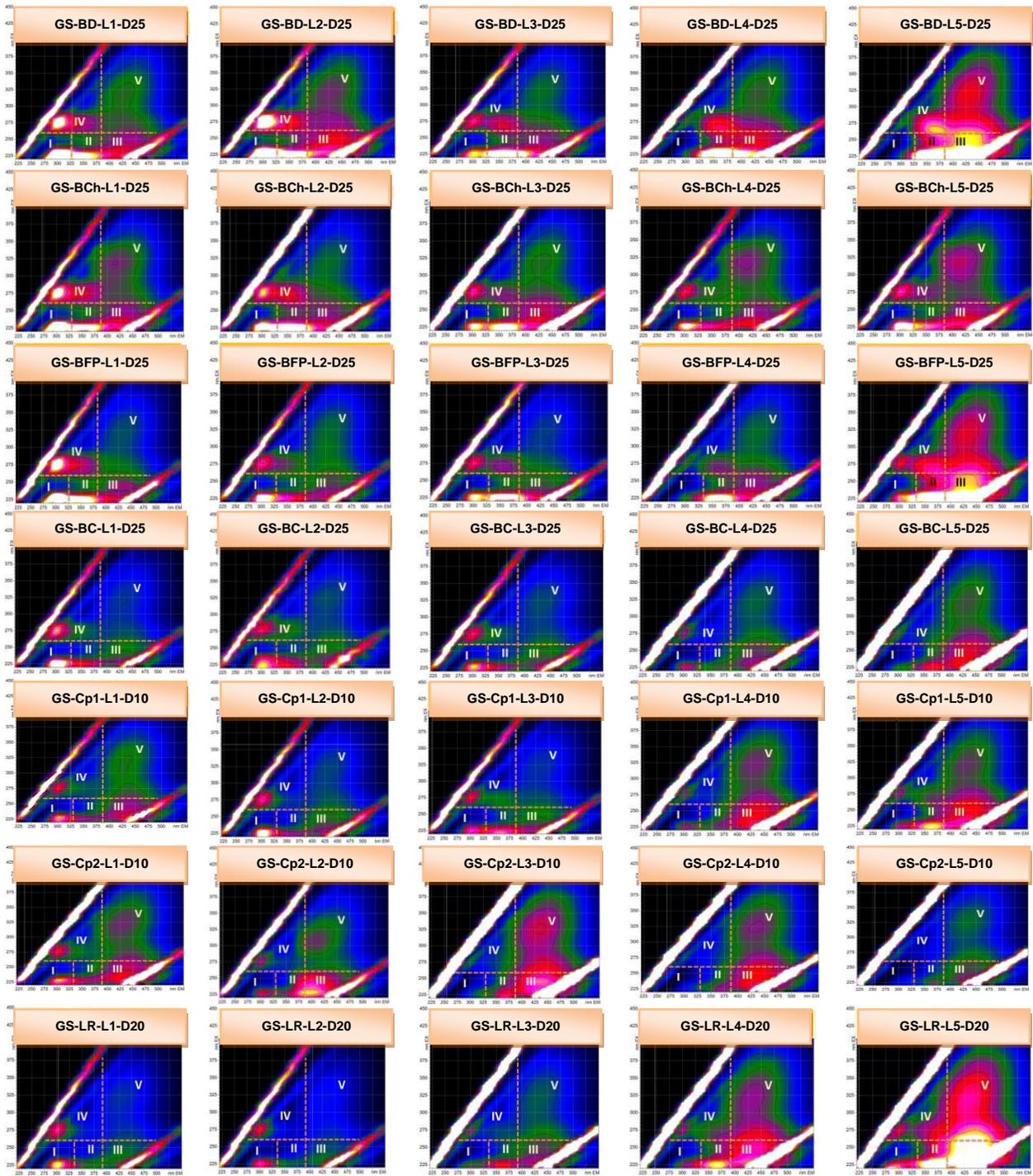


Figure 97: 3D spectra of leachates collected after each two days of leaching process from conventional sludge amended grass soil columns



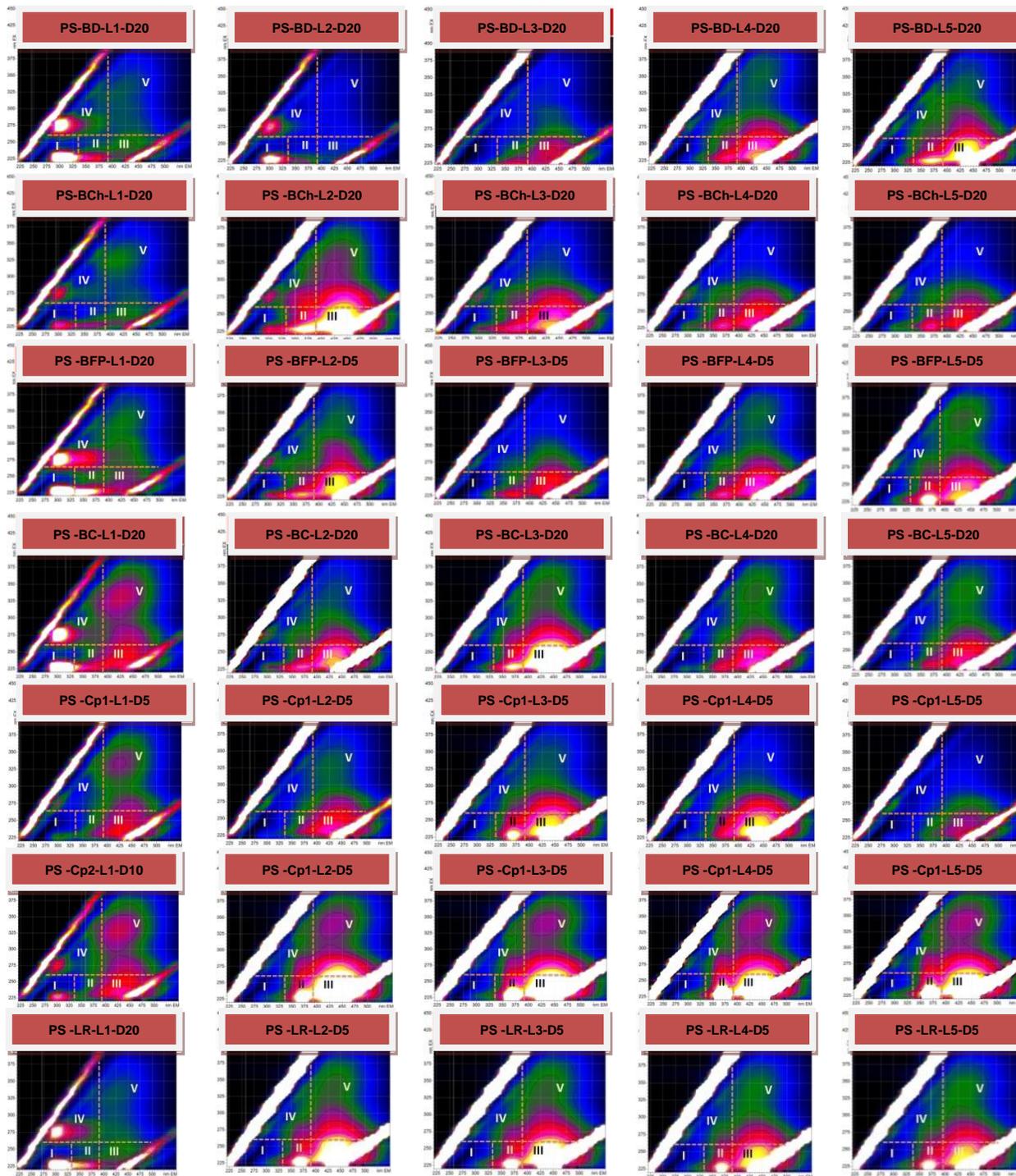


Figure 98 : 3D spectra of leachates collected after each two days of leaching process from conventional sludge amended paddy soil columns.



Appendices 4: Regulation for limit heavy metal concentration in drinking water (European countries) and surface water (Vietnam)

Table 43 : Regulation for limit heavy metal concentrations in drinking water according to Council directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption in European countries.

Heavy metals	Limit concentration	Unit
Arsenic	10	µg/L
Cadmium	5.0	µg/L
Chromium	50	µg/L
Copper	2.0	µg/L
Lead	10	µg/L
Mercury	1.0	µg/L
Nickel	20	µg/L
Selenium	10	µg/L

Table 44 : Regulation for limits heavy metal concentration in surface water according to TCVN 5942 – 1995 for surface water quality standard in Vietnams.

Heavy metal	Limit concentration		Unit
	A	B	
Arsenic	0.05	0.1	mg/L
Cadmium	0.01	0.02	mg/L
Lead	0.05	0.1	mg/L
Chromium (VI)	0.05	0.05	mg/L
Chromium (III)	0.1	1	mg/L
Copper	0.1	1	mg/L
Zinc	1	2	mg/L
Manganese	0.1	0.8	mg/L
Nickel	0.1	1	mg/L
Iron	1	2	mg/L
Mercury	0.001	0.002	mg/L
Selenium	1	2	mg/L

A: the standard was applied for drinking

B: The standard was applied for surface water





Comparaison entre des boues d'épuration issues de systèmes conventionnels ou granulaires pour l'adsorption des métaux et l'application au cuivre lors de l'épandage des boues sur des sols Français et Vietnamien,

Résumé :

L'épandage des boues d'épuration est un mode de valorisation à la fois écologique et économique pour autant que l'on maîtrise leur qualité et notamment la concentration des métaux lourds mobilisables. Le cuivre est une molécule très répandue, naturelle dans l'environnement, largement utilisée dans l'industrie et l'agriculture. En conséquence, les quantités de cuivre dans l'environnement ont augmenté, de sorte que ce dernier a été choisi dans cette étude comme représentant de la pollution anthropique pour évaluer sa mobilité en fonction de la qualité des boues épandues et des sols. 9 boues classiques et 2 sols (Sol d'herbe (de France) et sol de riz (du Vietnam)) ont été étudiés ainsi que des boues granulaires. Une méthodologie est proposée pour évaluer l'effet de la boue et de l'origine du sol sur la capacité de lessivage du cuivre lors de l'épandage des boues pour les activités agricoles. Les essais de désorption dans les colonnes de sol amendé par des boues ont montré que: a) quelle que soit l'origine du sol amendé, les boues de lit de roseaux (LR), séchées (BCD), centrifugées (BC) ont présenté une libération de Cu inférieure à 2% tandis que les boues de filtre-presse (BFP), digérées (BD), épaissies (BE) et calcaires (BCh) ont amélioré la disponibilité de Cu dans le sol; b) La lixiviation du cuivre dans le sol d'herbe (du France) amendé par des boues granulaires était supérieure à celle mesurée pour les échantillons classiques de boues; c) Dans le sol de riz (de Vietnam), l'épandage des boues granulaires a limité la désorption de cuivre par rapport aux échantillons de boue (BCh), de filtre-presse (BFP), digérée (BD) ou centrifugée (BC). Quatre mécanismes sont proposés pour expliquer le devenir du Cu dans les sols: 1) le contrôle; 2) le groupe de Cp1, Cp2, BC, LR and BCD; 3) le groupe de BCh, BD, BE, BFP et 4) le groupe de boue granulaire. Il est constaté que pour le sol de riz contenant une faible teneur en matière organique, l'application de boues granulaires ou d'autres types de boue tels que les boues compostées ou séchées peut non seulement augmenter la teneur en éléments nutritifs, mais aussi réduire la capacité de lixiviation du cuivre.

Mots-clés [Procédé conventionnel de boues activées, procédé de boues granulaires, métallelement de traces, épandage, sol d'herbe, sol de riz, essai discontinu, essai de colonne]



Comparison between granular and conventional activated sludge for trace metals sorption/desorption ability. Case of copper for land spreading application in France and in Vietnam.

Abstract :

The spreading of sewage sludge is both ecological and economical insofar as their quality is controlled and in particular the low level of the heavy metals available. Copper is a very common substance, naturally occurring in the environment, widely used in industry and agriculture. As a result, quantities of copper in the environment have increased, so it has been selected in this study as representing human-induced pollution to assess its mobility in relation to the quality of sludge and soil. 9 different sludges and 2 soils (grass soil from France and paddy soil from Vietnam) were studied as well as granular sludge. A methodology was developed to evaluate the effect of sludge and soil origin on copper leaching ability during sludge application for agricultural activities. Desorption tests in soil-amended sludge columns showed that: a) Whatever the origin of soil, the centri-dried (BCD), centrifuged (BC), reed-bed (LR) sludges presented a Cu release lower than 2% while filter-pressed (BFP), digested (BD), thickened (BE) and limed (BCh) sludge amended soils increased Cu availability in soil; b) the copper leaching of granular sludge amended grass soil was higher than those measured for conventional sludge samples; c) in paddy soil, landspreading of granular sludge limited the Cu desorption compared to limed (BCh), filter-press (BFP), digested (BD) and centrifuged (BC) sludge samples. Four mechanisms groups are proposed to explain the fate of Cu in soils: 1) the control; 2) the group of Cp1, Cp2, BC, LR and BCD; 3) the group of BCh BD BE and BFP, and 4) the group of granular sludge. It is found that paddy soil containing low organic matter, application of granular sludge or other types such as composted or dried sludge can not only increase the nutrient content but also reduce the leaching capacity of copper.

Keywords: [conventional activated sludge process granular sludge process, trace metal element, landspreading, grass soil, paddy soil, batch test, column test]

