Chapter 4

Engineering of trace-element supplementation

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ABSTRACT

Anaerobic digestion industries need to achieve higher performance and strive harder to play a key role in the green future of the energy sector. The importance of trace elements (TE) in the welfare of anaerobic bioreactors must be taken into account by the stakeholder/user to achieve these objectives. However, the implementation of a TE strategy is often stopped by its complexity, a lack of resource and the economic reality of a full-scale operating plant. The aim of this chapter is to support the translation of academic research findings to the engineering and operating of full-scale plant. Management tools have been developed to help operator and stakeholder in their TE assessment of their anaerobic digestion (AD) plant and suggest potential strategies to overcome deficiency. It is essential to understand the key elements of the AD system when developing the TE strategy. Feedstock

doi: 9781789060225_0073

is the sole natural provider of TE to the AD and determines the matrix inside the reactor. Reactor design and operating conditions fix the chemical environment that governs the behaviour and availability of the TE while controlling the TE need for bacterial population.

KEYWORDS: anaerobic bioreactor, design, full-scale, management tools, operating condition, trace element supplementation

4.1 INTRODUCTION

Energy from anaerobic digestion (AD) contributes towards the targets for renewable energy production and greenhouse gas mitigation in many countries. European countries have developed modern biogas technologies and competitive national biogas markets throughout decades of intensive research and technical development (Al Seadi *et al.* 2008). This has been achieved through support schemes, national policies, the efforts of private companies and stakeholders, and high quality research in universities/research centres.

Anaerobic digestion developed at first as a sludge-processing technology, aiming to reduce sludge volume and pathogen content prior to its disposal. The process design was simple and the biochemical knowledge minimal. In the latest four decades, anaerobic digestion has evolved into a process able to produce/recover energy through the production of methane. Research studies have been conducted to enhance the rate of biogas production, increase the speed of conversion of various feedstocks and test the ability of anaerobic digestion to treat feedstock other than sewage sludge (agricultural residue, food waste and many others). The expansion of the AD technology, through this new vector, happened throughout Europe, particularly in Germany, Italy and the United Kingdom. The number of biogas and biomethane plants at the end of 2015 were over 17,000 and 450, respectively. The generation of biogas by anaerobic digestion technology was over 18,000 million m^3 in 2015, representing 4% of the biogas share in the natural gas use (Scarlat *et al.*, 2018). The use of the biogas was divided between electricity generation (61,000 GWh) and heat production (130,000 TJ). In 2015, the European biogas sector provided 66,200 jobs representing 6% of the total jobs within the renewable energy sector; the biogas turnover in 2015 was estimated to be 6 bn € (EurObserv'ER, 2015).

Commonly, biogas plants produce electricity and heat by the combustion of biogas on-site in combined heat and power units, but anaerobic digestion can also be a source of biomethane. The purified biogas can then be injected to the gas grid (1.4 million m^3 in 2015; Scarlat *et al.*, 2018) or used as a biofuel for transportation. Biogas and biomethane are storable energy sources and they can balance the intermittent supply of other renewables such as wind and solar power (Mauky *et al.*, 2016).

Although anaerobic digestion has been adopted widely throughout the agricultural and wastewater sectors to recover bioenergy for waste, the process cost-effectiveness has considerably hindered its expansion in the energy market. AD operators often rely heavily on government subsidies in order to remain operational and compete with other sources of energy. However, government subsidies (green energy subsidies and feed in tariffs) have been reducing dramatically over the past few years, i.e. by 40% in the case of UK, jeopardizing its future growth.

If anaerobic digestion wants to play a key role in the energy of the future, it needs to become economically sustainable without any government incentives; one way to achieve this is through better and quicker degradation of the feedstock. Studies have been conducted on the physical parameters of AD to improve mixing, heating and pre-digestion treatment. AD comprises a series of sequential and interdependent microbiological reactions and it is thus necessary to ensure that the microbial community underpinning the process is as active as possible and performs optimally. Several important factors influence microbial growth and activity, including ideal conditions of pH, temperature and redox potential; carbonaceous substrates; macronutrients, such as nitrogen and phosphorus; and micronutrients i.e. trace elements (TE). The balanced availability of various nutrients coupled with the provision of ideal growth conditions is essential for anaerobic digesters. Disruptions to one or more of these factors may disturb the activity of specific groups of microorganisms and, thus, impair digester performance.

The supplementation of trace elements has a strong influence on microbial metabolism and can inhibit activity in cases of excessive concentrations or of low bioavailability. Studies showed that biogas production can be enhanced by 15–30% with effective supplementation of TE. If the energy output from the UK AD sector, as an example, could be boosted by 20%, this would generate up to 2.1TWh of additional energy. The extra generation from the same infrastructure would be able to provide green energy for 460,000 households (equivalent to a city of the size of Birmingham, Naples or Köln), generating an extra revenue of £230 million per annum. Environmental benefits will be a reduction in CO_2 emitted and the volume of biosolids for land spreading or incineration.

Engineering research needs to implement the findings from the previous chapters (TE speciation, microbial interaction) to industrial digesters. The main challenge is to scale-up the supplementation of TE on a full-scale anaerobic digester in a cost effective manner. The accurate work done in laboratory-controlled environments cannot be directly translated to a full-size anaerobic digester as the operational conditions are not completely known or can vary. The quality of the feedstock, mixing system or even the pH can vary and influence the TE requirement of the microbial community. Each digester is unique and will require a specific solution, however, using literature on previous studies and expert knowledge, the development of an efficient dosing strategy or management tool is feasible, taking into account the reactor specificity, the anaerobic digester operating conditions and the type of feedstock.

4.2 MANAGEMENT TOOLS

Process monitoring helps to understand the biochemical and physical-chemical processes occurring within a biogas plant, while helping to maintain the process stability. Thus, key parameters have been identified as early indicators of process imbalance and prediction models have been applied in practice to simulate anaerobic bioreactor performance (Drosg, 2013; Soren & Nelles, 2015). Control objectives to optimize the anaerobic fermentation are shifting from the regulation of key variables (measured on-line like temperature, mixing intensity, loading rate) to the prediction of overall process performance (generally off-line measurements like chemical analysis). Human operation is often included in the management loop when taking decisions to modify the steady state of the process. Trace elements are one of the key parameters that can influence anaerobic bioreactor performances and recent studies show the starvation of TE could be a rate-limiting step in the process (Qiang *et al.*, 2012).

The decision on whether TE supplementation of AD is required and, if it is the case, how to approach the dosing process requires the consideration of different scenarios and required outcomes. A management tool diagram has been developed covering the different scenarios typically encountered while operating large scale AD (Figure 4.1). The diagram is divided into three sections: evaluation (top), characterization (middle) and supplementation (bottom).

The first (top) area is the evaluation of general AD performance. The reasons behind an underperforming digester can be multiple. Data collection of the operating conditions, feedstock and digester parameters will allow a global assessment of the digester well-being and capabilities. TE limitation needs to be considered if no obvious causes have been found, such as temperature fluctuations, presence of inhibitor or salinity.

The second (middle) area focuses on TE requirements. Extensive elemental analysis of the feedstock is crucial to spot potential deficiency in key TEs. Analytical methodologies consider feedstock characteristics (TS, VS, BMP – biochemical methane potential) and residual methane potential to evaluate the performance of full-scale biogas plants (Ahlber-Eliasson *et al.*, 2017; Ruile *et al.*, 2015).

The third (bottom) area relates to the supplementation of TE if required. Two main directions can be taken to increase the bioavailability of identified deficient TE. The first option is to supplement a concentrated solution of TEs mixed with the feed or directly to the anaerobic digester. This is often recommended if the anaerobic digester is showing sign of struggling with potential failure. A high pulse addition of the TE cocktail might quickly recover the AD to normal performance. In a non-urgent case, the work prior to starting the TE supplementation should focus on the concentration required, the type of compounds used (salt or organic ligand), and the dosing system (continuous or pulse). The second option is to adapt the current system to increase the bioavailable section of the TE without any supplementation.

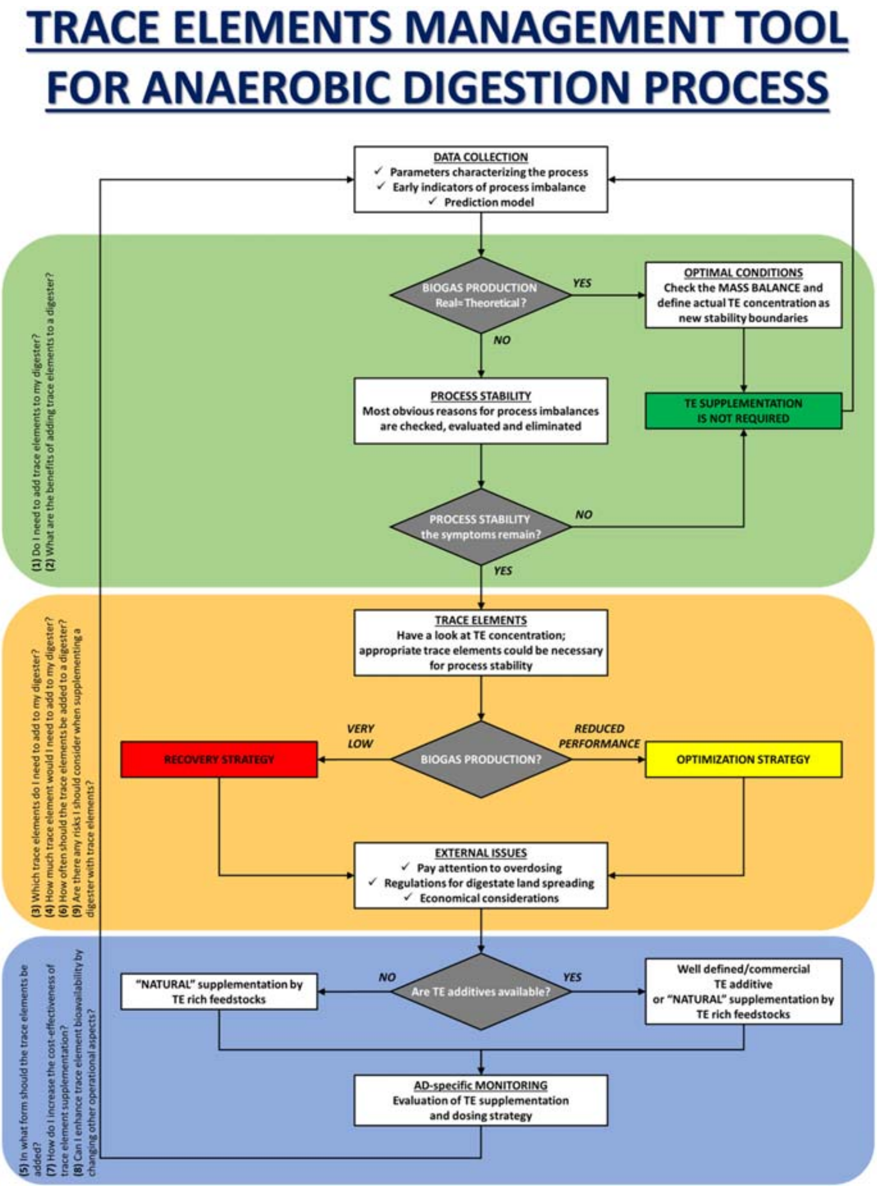

Figure 4.1 Trace-elements management tool.

4.3 INFLUENCE OF REACTOR TYPE ON TE SUPPLEMENTATION

The type of reactor plays an important role in TE supplementation as it defines the microbial community and its requirement. The approach on the best way to supplement TE will be highly dependent on the reactor used. As an example, the threshold for TE deficiency in a feedstock will vary if the system is a batch or continuous reactor.

In a batch system, the amount of TEs supplemented should be tailored to biomass/feedstock concentration and bioavailability. The total content of TE introduced in the system through the inoculum and feedstock will be identical until the end of the reaction, and the focus should be on the amount that will be available to microorganisms during the whole process. The TE/volatile solid ratio will increase as the organic matter decreases over time (Dong *et al.*, 2013). The speciation of each TE will move towards a pseudo-steady state during the whole process, influencing the bioavailable fraction. Roussel (2013) demonstrated that the behaviour of cobalt, nickel and zinc was different when supplemented as EDTA complexes. Nickel remained in the liquid fraction while zinc was precipitated in the first days of the experiments. With cobalt, the transfer to the solid fraction was slowly observed during the 30 day experiment and was dependent on the speciation of iron in the digester. In another example, Cai *et al.* (2018) demonstrated that the fractionation iron in the AD of rice straw, during the 50 days of batch mode experiment, remained unchanged. Despite the total concentration of iron varying from 9.3 to 13.2 g L^{-1}, the authors found that the iron bioavailability was low and that residual fraction was the most representative fraction, accounting for 97–98% of the total concentration. The authors also demonstrated that the supplementation of iron improved the reactor performances.

In a continuous system, the amount of TEs inside the system will be dependent on the capacity of the system to retain the TEs, particularly if the hydraulic retention time (HRT) is different to the sludge retention time (SRT). The total concentration and the bioavailability fraction will be highly dependent on the feedstock TE content and their speciation inside the reactor. An unbalanced system might lead to a TE deficiency or a toxic accumulation. González-Suárez *et al.* (2018) observed the TE deficiency in semi-continuous reactors during the digestion of maize straw. The authors operated two SCSTR reactors, a control reactor without metal addition and a reactor with TE supplementation, both with OLR varying from 0.5 to 2.0 gVS L^{-1} d^{-1}. The control reactor showed a loss of 28% in Fe and of 44% in Co total concentration inside the reactor after 96 days leading to lower reactor performance. The methane yield of the TE supplemented reactor was improved by 30%, compared with the control reactor. The trace elements, from a natural clay-mineral mixture source, were added at a concentration of 1 g L^{-1} once a week during 28 days.

In a single stage reactor, the entire full microbial consortium responsible for the AD process is subjected to the same system conditions. In perfectly mixed flow reactors, such as continuously stirred tank reactor (CSTR), the composition of the reactor is uniform and only varies over time. In a plug-flow reactor, the composition of the media varies through the reactor height (Levenspiel, 1999), thus the microorganisms located in a posterior position are subject to a concentration of TE where their bioavailability is dependent on phenomena occurring in the anterior portion of the reactor. Most full-scale reactors are in a flow pattern intermediate between perfectly mixed and plug-flow.

The sorption mechanism of TEs from the soluble to the solid phase is determinant in the retention of the TE in the anaerobic reactor. In this sense, reactors with granular sludge or support material for biomass retention allowing a higher SRT than HRT guarantee longer TE residence time in their system. The TE uptake is affected by biomass concentration in the reactor and the sorption kinetics. Long SRT should then limit the risk of washout, which is a constant challenge in a continuous system. However, a slow mass transfer of TE inside the biofilm might limit their availability to the microorganisms compared with suspended biomass.

Two UASB reactors fed with methanol were assessed by van der Veen *et al.* (2007), both supplied with TE, but one without addition of Co in the substrate and the other deprived of Ni. The authors evaluated the bioavailability of the TE according to sequential metal extraction (SME) using the Tessier modified method. The SME divides the TE into four fractions, in order of their binding strength. The author found that Co did not exhibit losses in the reactor deprived of cobalt addition; it was mostly present in a strongly bound fraction, suggesting excellent retention of this metal in the UASB reactor. On the other hand, Ni was lost relatively quickly from the UASB reactor and, correspondingly, it was extracted at 72% in the most loosely bound fraction, with only 18% in a strongly bound fraction. A submerged anaerobic membrane bioreactor (SAMBR) is more efficient in terms of biomass retention than the UASB reactors since, due the presence of the membrane, the biomass can form a biofilm, leading to higher solids retention time (SRT). Thanh *et al.* (2017) found that the TE retention during supplementation in SAMBR varied in comparison to a UASB. Zinc and iron were better retained in SAMB, while nickel and cobalt were retained to a lesser extent. The authors also demonstrated that the losses occurred when TE had a high content in loosely bound fractions.

Voelklein *et al.* (2017) carried out a single- versus two-phase system experiment to equate the TE requirement to the specific microorganisms involved in the different stages of the AD. TE supplementation was evaluated in a single- and two-stage system for digestion of food waste. Both systems failed when exceeding the OLR of 2.0 g VS L^{-1} d^{-1} and were restored after the addition of Co, Fe, Mo, Ni and Se. The specific methane yield rose to the same levels as before for both systems, with the single-phase presenting higher values. The requirements of a two-stage system, with supplementation only in the second reactor (methanogenic), was optimized to reduce the TE concentration needed since the Archaea community is more sensitive and responsible for the major stage in the AD.

4.4 INFLUENCE OF OPERATIONAL CONDITIONS ON TE SUPPLEMENTATION

Operational conditions play an important role in the quantity and quality of TE supplementation considering they affect the anaerobic microbial community dynamics (Fontana *et al.*, 2016; Xu *et al.*, 2018). In this section, the role of TEs in association with organic loading rate, temperature, pH and hydraulic retention time (HRT) are reviewed.

4.4.1 Organic loading rate

Results and conclusion from several literature studies demonstrate that the maximum organic-loading rate supported by the digester is dependent by the bioavailability of TEs. TE supplementation might be required to increase the ORL while keeping the process in balance.

Semi-continuous digestion tests were conducted in 2 L stirred tank reactors at 38°C fed with cattle slaughterhouse wastewater; increasing the OLR step-wise from 0.3 to 2.76 g COD L^{-1} d^{-1} with a corresponding decrease in HRT throughout 170 days of monitoring (Schmidt *et al.*, 2018). The TE solution consisting of Fe, Ni, Co, Mn, Mo was added daily to three digesters to reach optimal concentrations (Lemmer *et al.*, 2008) and the remaining three reactors were used as controls. The addition of TEs resulted in enhanced degradation efficiency, increased biogas production and an improved process stability. Higher OLR and lower HRT were achieved with TEs supplementation in comparison to the control digesters; control reactors exhibited a process failure at OLR 25% lower than the maximum investigated in the test and that achieved with TE supplementation (Schmidt *et al.*, 2018).

In another case, two continuously stirred tank reactors with 8 L volume fed semi-continuously at 39°C with a substrate mixture consisting of manure and industrial waste were used to evaluate the effects of TE addition in a 104 day test. The OLR was 2.4 kg VS^{-1} m^{-3} d^{-1} in the first 66 days and it was increased to 3.3 kg VS^{-1} m^{-3} d^{-1} at day 67, until the end of the test. The first reactor was used as control where only iron was supplied while the second reactor worked with iron and TEs, the composition of which was set to reach an additional 0.2 mg kg^{-1} Co and Ni concentration in the digestate (Nordell *et al.*, 2016). This experiment clearly demonstrated that the supplementation of TEs was necessary to achieve a fully stable and optimal process as the reactor supplied with TEs showing decreased volatile fatty acid (VFA) concentrations (−89%), greater biogas production rate (+24%) and better biogas yield (+10%) at upper OLR (3.3 kg VS^{-1} m^{-3} d^{-1}) if compared with the control reactor. Furthermore, the foam occurring in the control reactor at high OLR was probably due to the accumulation of long chain fatty acids or other organic intermediary, sign of an unstable process (Nordell *et al.*, 2016). The TE supplemented reactor did not suffer from foaming.

4.4.2 Temperature

Two main process temperatures are used in anaerobic fermentation and digestion systems, mesophilic conditions, 35–40°C and thermophilic conditions, 50–65°C (Bischofsberger *et al.*, 2004; Gerardi, 2003). However, the temperature range between 40°C and 50°C is also important to take into consideration because of the self-induced temperature increase sometimes observed during anaerobic digestion. This phenomenon, occurring at full-scale, can cause severe process disturbances (Lindorfer *et al.*, 2008). The digester temperature has a direct influence on the microbial community present in the reactor and so the TE requirement. Moreover, the chemical reactions determining TE speciation are also temperature dependent.

A well known advantage of thermophilic systems is higher conversion rates or higher OLR in comparison to mesophilic systems (Ahn, 2000). The higher conversion rates could be due to quicker microbial growth rates because thermophilic methanogens in general have a faster duplication rate if compared with mesophilic methanogens (Borja *et al.*, 1995). Several studies revealed an increase in degradation rates for thermophilic processes, compared with mesophilic system. This increase cannot be associated with the catalytic rate of enzymatic reactions as mesophilic and thermophilic enzymes are fully comparable at their optimum temperatures, regarding structure, catalytic route and thermo-sensitivity (Elias *et al.*, 2014; Zavodszky *et al.*, 1998). The effect on enzyme production under sub-optimal growth conditions is unknown but if enzyme production is assumed suboptimal in both cases, the amount of TEs that are needed for a thermophilic system to be optimised could, in general, be considered higher compared with similar mesophilic system (Hendriks *et al.*, 2018).

Literature suggests different nutritional requirement for various temperature ranges. Minimum requirements for iron, nickel, cobalt, and zinc in thermophilic methane fermentation from glucose has been observed to be ten times higher than those necessary for mesophilic anaerobic fermentation from acetate, indicating a possible decrease in bioavailability or increase in nutrient requirements at thermophilic temperatures (Takashima & Shimada, 2004).

In a laboratory-scale study with 5 L digesters that work at high ammonia concentrations (0.5–0.9 g NH_3 L^{-1}) with the OLR set to 2.3 g VS L^{-1} d^{-1} and an HRT of 30 days, the TEs supplementation containing Fe, Co, Ni, Se, W showed differences even within the mesophilic range. An increase in the operating temperature of only 5°C (from 37°C to 42°C) without TE addition had no impact on methane yield, but resulted in a large amount of volatile fatty acids accumulation, particularly propionate, which imbalanced the process at 42°C. The TE addition at 42°C resulted in 12–23% increased methane yield and an improvement of the degree of degradation with a decrease in propionate concentration from 4 g L^{-1} to below 0.1 g L^{-1} (Westerholm *et al.*, 2015).

In full-scale anaerobic digesters the individual temperature depends on plant specific parameters such as feedstock type and quantity, self-heating, insulation potential and availability of cooling system. The seasonal variation of the external temperature and other factor complicates the maintenance of the optimal temperature (Lindorfer *et al.*, 2008) and those fluctuations affect the process stability due to the temperature sensitivity of methanogenic *Archea* (Speece, 1996). TEs supplementation could be a reasonable strategy to reduce the negative impacts of operating temperature changes.

4.4.3 Two phase anaerobic digestion/pH role

The physical separation of hydrolytic/acid-producing and methane-producing microrganisms in two different reactors, where optimum environment conditions for each group of organisms would be provided, is considered an interesting technology for overall process improvement. The hydrolytic/acidogenic reactor is often operated under moderately acidic conditions (pH 5–6) at a short HRT (<5 days) (Pohland & Ghosh, 1971; Ponsà *et al.*, 2008).

Changes in pH and HRT can not only affect the performance of the anaerobic process, it can also radically affect the metal speciation of TMs with interrelated effects (Thanh *et al.*, 2016). Generally, literature studies showed that increase in pH led to the transformation of TEs from available mobile fractions to stable organic forms reducing their bioavailability. In contrast, a reduction in pH results in the dissolution of TEs into the liquid medium, shifting them towards more bioavailable fractions (Dong *et al.*, 2013; Lopes *et al.*, 2008). Taking in consideration other parameters, the redox reaction between Fe and S lead to iron sulphide (FeS) formation and the interaction between precipitated FeS and TEs resulting in the formation of insoluble FeS–metal complexes (Dong *et al.*, 2013) with limited bioavailability for hydrolytic bacteria.

Recent studies investigated the determination of TEs in the first phase of anaerobic digestion, where hydrolysis and acidification are considered together due to their close connection (Burgess & Pletschke, 2008; Frey & Hegeman, 2007): TEs involved in hydrolytic enzyme reaction mechanism include Mg, Zn, Ni, Co, Se, Mo, and Fe.

A substrate consisting in maize silage (75%), grass cuttings (15%), winery by-products (5%), cow manure (2.5%), and other bio-wastes (2.5%) was used in anaerobic digestion experiments with batch reactors to evaluate the influences of TEs concentration and composition on the hydrolysis rate. Results showed that at high volatile fatty acids concentration (200 mmol/L) the increase in Co concentration is required to ensure good hydrolysis of the substrate and its conversion to VFA. Moreover, the simultaneously increasing of Co and Se concentrations will improve substrate hydrolysis and acidification rate (Ezebuiro & Körner, 2017). This study concluded that the original composition and concentration of TEs in substrates could be unbalanced and lower than the required level for continuative substrate hydrolysis; different TEs combination addition, i.e. Co and Se, could be beneficial for the process.

4.4.4 Hydraulic retention time

Studies on the relationship between HRT and TEs supplementation in anaerobic processes are very limited. The evaluation of Cu and Zn distribution in swine wastewater in an up-flow biodigester at different HRTs reported a decreased metal-retention capacity of the bioreactors when the HRT was reduced from 18 days to 7.5 day (Cestonaro do Amaral *et al.*, 2014). This finding is important for the operation of biogas plants in two-phase anaerobic digestion systems: the acidogenic reactors with short HRT could need a higher concentration of TEs to compensate for the fraction of the metals which become less bioavailable (Thanh *et al.*, 2016), primarily when the hydrolysis rate represents the limiting factor of the process.

The removal of VFAs from the acidogenic reactor to feed the second stage methanogenic reactor is an important parameter in a two-phase system to control VFAs concentration and pH in the first stage, as well as the OLR in the methanogenic reactor (Cysneiros *et al*, 2012). In full-scale digesters, the partial recirculation of the effluent from the methanogenic stage to the acidic stage can help to buffer the rapidly produced VFAs, maintain a suitable pH and enhance the process to overcome the bacterial wash out problem (Zuo *et al.*, 2013). At the same time the recirculation can guarantee a partial recycling of TEs for the system, limiting the lack of nutrients that occur in the acidogenic reactor with short HRT. A proper management of periodicity and volume of recirculation with its nutrient recycling leads to the creation of favourable conditions for microbial growth and nutrient transport for both acidogenic and methanogenic phases.

Many operational parameters have been confirmed to be associated directly or indirectly with anaerobic microbial evolution and methane generation. They are often set to drive the fermentation towards one direction rather than another, sometimes with the purpose of recovering an unbalanced system, or in other cases to optimize the process. These alterations in many cases bring unpredictable physico-chemical variations of the environment and shifts in microbial population that affect the nutrient requirement of the digester. The metal–biomass interaction and sulphide chemistry trigger reactions of precipitation, co-precipitation, adsorption and uptake that influence the bioavailable fraction of TEs which can be utilized in the anaerobic digestion process. At full scale, the awareness that some nutrients are retained in the digester and others will be released with the effluent digestate according to applied operational conditions, and that the metals could be more or less bioavailable depending on their speciation has to be clear. Improvement in the knowledge of these mechanisms will lead to tailor-made TE formulations and dosing strategies developed to have a specific concentration of TEs in their bioavailable form to best fit each anaerobic digestion process with its operational conditions.

4.5 IMPORTANCE OF FEEDSTOCK CHARACTERIZATION ON TE SUPPLEMENTATION

Trace elements are usually supplied with feedstock to the biogas plants, being necessary for its evaluation to ensure a stable and optimal anaerobic digestion process (Schattauer *et al.*, 2011). The metabolic effect of the trace elements is dependent on the concentration of trace elements in the feedstock, but also on the availability and speciation of the trace elements (Demirel & Scherer, 2011). Complexation reactions (in the liquid phase or solid phase) play an important role in bioreactors making a particular trace element either more or less bioavailable (Fermoso *et al.*, 2015). Other main mechanisms involved in the bioavailability are the chelation of metals, the ion exchange, the adsorption, the inorganic micro-precipitation and the translocation of trace elements into the microorganisms (Zandvoort *et al.*, 2006).

The shortfall supplementation of trace elements can results in the destabilization of the process due to their necessary role as cofactors in different metabolic pathways (Fermoso *et al.*, 2015; Matheri *et al.*, 2016). An excess of trace elements in the supplementation could entail their accumulation up to inhibitory concentrations (Fermoso *et al.*, 2015). Another concern of an excessive supplementation would be an undesirable accumulation of trace elements in the final digestate, which could limit land application (Serrano *et al.*, 2014). Moreover, an excess of trace-element supplementation is costly. Therefore, it is very important that the correct use of the management tools are used, as described in section 4.3. A detailed evaluation and characterization of the anaerobic system can optimize the supplementation of the trace metals, avoiding the previously described undesirable effects.

As the feedstock is the primary source of trace elements, and many other compounds that could influence the chemical bioavailability of the trace elements, it is important to carry out a deep characterization of the feedstock. Although each feedstock has its own character, four different types of feedstock can be defined that will influence trace-element bioavailability in digestion: sulphur-rich, phosphate-rich, lipid-rich and lignocellulosic waste.

4.5.1 Sulphide-rich feedstock

Sulphur is an essential macronutrient for the growth of methanogenic microorganisms (Mountfort & Asher, 1979). However, the presence of sulphur compounds in the reactor could affect the bioavailability of trace elements, even at very low concentrations (Gonzalez-Gil *et al.*, 2003; Zandvoort *et al.*, 2006). Most trace elements predominantly react with sulphide to form insoluble salts and sulphide concentration is a determining factor in the TE speciation and bioavailability (Fermoso *et al.*, 2009; Roussel & Carliell-Marquet, 2016). Typical sulphide-rich feedstocks are pig slurry, algae (fucoidan content) and acid-mining drainage and examples of TE concentrations are shown Table 4.1.

One of the main factors affecting trace-elements bioavailability is the presence of sulphate-reducing bacteria (SRB), which reduce the sulphate to sulphide in anaerobic conditions (Chen *et al.*, 2008; Dall'Agnol & Moura, 2014). The precipitation of trace elements in the form of metal sulphides enhances the retention of the trace elements in the digesters, although these trace elements may be no longer bioavailable for the uptake by microorganisms (Zandvoort *et al.*, 2008). Studies demonstrated that presence/addition of sulphide reduced the bioavailability of several TEs (such as zinc, iron, copper) by a translocation from the carbonate to the sulphide fraction (Zandvoort *et al.*, 2006, 2008). Therefore, the monitoring of the trace elements concentration based on the total concentration could not be representative enough of the requirements of the microorganisms, therefore the monitoring of the soluble concentration or, if possible, a more detailed fractionation study is recommended (Pinto-Ibieta *et al.*, 2016).

4.5.2 Phosphate-rich feedstock

Phosphorous is a basic nutrient for the growth of the anaerobic microorganisms and it also plays a key role in the immobilisation of the biomass on mineral particles (Chen *et al.*, 2008). The anaerobic digestion of phosphate-rich feedstock can result in high concentrations of soluble phosphate in the digesters (Carliell-Marquet & Wheatley, 2002). Sewage sludge is the main phosphate-rich feedstock due to chemical phosphorus removal processes occurring in wastewater treatment plants. The concentration of phosphate in the sludge can be increased 10-fold or more (Roussel & Carliell-Marquet, 2016). Fishery waste can also have a high phosphate content and examples of these feedstock are shown in Table 4.2. The increase in the concentration of phosphate has a direct effect on the concentration and bioavailability of others cations, such as calcium, magnesium and manganese, and hence, of the trace elements (Carliell-Marquet & Wheatley, 2002; Marti *et al.*, 2008; Muhmood *et al.*, 2018).

Roussel and Carliell-Marquet (2016) demonstrated that iron speciation was influenced by the presence of high phosphate concentration with the formation of vivianite, especially in sewage sludge. The presence of iron as vivianite increased its mobility of iron in the sludge in comparison with iron-sulphide salts. This shift on the iron speciation might change other TEs bioavailability through co-precipitation or chemical reactions (Roussel, 2013).

Table 4.1 Trace-elements composition of different sulphide-rich feedstocks.

		S ppt	N ppt	P ppt	K ppt	Al ppt	Cu ppm	Zn ppm	Mg ppm	Fe ppm	Mn ppm	Co ppb	Ni ppb
Marcato *et al.* (2008)	Pig slurry	8.1	2.8	29.5	37.4	0.868	590	1500	14,200	2500	0.629		
Radis Steinmetz *et al.* (2009)	Pig slurry				0.925	0.146	34.8	43.9	17.4	171	386	251	244
Formentini *et al.* (2017)	Pig slurry		3.7	0.6	1.7		21.0	43.0					
Ometto *et al.* (2018)	Algae (*S. latissima*)	2.66		0.46	7.7	130	6.02	11.9	1680	476	3.22	<420	840
Barbot *et al.* (2015)*	*Laminaria japonica* Waste	8.9	20.0	2.1	89.9		5	28	6800	3440	150	1500	3000
Migliore *et al.* (2012)	Algae	1.82	1.74				9.8	88		3920			4.3
Li *et al.* (2014)	Synthetic saline wastewater	0.6			1.46		0.024	0.024	0.009	0.014	0.042	10.8	22.6
Ueki *et al.* (1988)	Acid main drainage	157					550	9100		9.704	13.8		
Smyntek *et al.* (2018)	Acid main drainage	222	<1	<1		37.4				19,800	2.6		
Fernández-González *et al.* (2018)	Acid mining effluent	2.7	0.5	<0.001			5.23	20.91			8.53		14,204
Moraes *et al.* (2015)	Sugar beet vinasse	1.76	26.4	0.13	11.2	0.21	0.41		0.05	15.32	9.75	<0.5	660
García-Depraect *et al.* (2017)	Vinasse	0.286	0.188	0.341	0.371		<1.0	2.3	136.2	14.3	<1.0	<1.0	<1.0

*Dry basis.

Table 4.2 Trace-element composition of different phosphate-rich feedstock.

		S ppt	N ppt	P ppt	K ppt	Al ppt	Cu ppm	Zn ppm	Mg ppm	Fe ppm	Mn ppm	Co ppb	Ni ppb
González *et al.* (2017)	Sewage sludge		33.4	97.8	3.81		261	456					19,000
Serrano *et al.* (2014)	Sewage sludge		7.94	4.23			95	167					64,000
Martí *et al.* (2010)	Sewage sludge		0.104	0.071	0.072				60				
Wan *et al.* (2011)	Thickened sewage sludge		4.0	1.231	0.32		17.8	54.5	291	628	54.3	<0.2	5680
Muhmood *et al.* (2018)	Poultry slurry		4.5–5	0.22	2.58		1.69	2.91	0.014				550
Wang *et al.* (2016)	Acido-genic effluent	0.06	0.7	1.246	0.121	1.53	1.00	0.28	90.45	12.39	<0.10		<200
Bohutskyi *et al.* (2015)	Waste-water media	0.029	0.023	0.006			0.064	0.035	11	1.0	0.19	1.8	
Alvarenga *et al.* (2017)	Primary sludge		0.14	0.07	0.02	354	0.448	1.416	11.8	44.25	0.885		
Goddek *et al.* (2016)	Aqua-culture waste-water	0.199	0.057	0.192	1.44				396				
Vivekanand *et al.* (2018)	Fish ensilage	4.90	64	9.2	7.95	135	11.0	0.098	1200	420	10	60	510
Madariaga & Marín (2017)	Fish farming sludge		9.62	7.02			3.77	140					
Andreji *et al.* (2006)	Fish muscle						0.128	1.01		1.64	0.056	24	14
Nges *et al.* (2012)	Fish waste	1.8	23.8	7.7	1.6		0.9	88	380				60

Magnesium is one of the most affected cations by the high phosphate concentration due to its precipitation in the form of struvite and/or other salts (Muhmood *et al.*, 2018; Wang *et al.*, 2016). The precipitation of struvite (magnesium ammonium phosphate hexahydrate) has been widely proposed as a phosphorous recovery and valorisation technique from phosphate-rich feedstock given its potential as fertilizer (Yilmazel & Demirer, 2013). Other trace elements can precipitate associated to the precipitation of the struvite. For example, Muhmood *et al.* (2018) reported the removal of 40, 45, 66, 30 and 20% of zinc, copper, lead, chrome or nickel, respectively, during the precipitation of struvite in a reactor treating poultry slurry. Similar results were also reported by Liu *et al.* (2011) at the struvite recovery from swine wastewater. The co-precipitation of the trace elements and joint extraction with the struvite could result in a deficit of trace elements in the long-term operation of the anaerobic processes. Even if the phosphate is not recovered in the form of struvite, high concentrations of phosphate have been related to a strong decrease in the bioavailability of magnesium and manganese (Carliell-Marquet & Wheatley, 2002). Therefore, trace-element supplementation for anaerobic digesters treating phosphate-rich feedstock should take in account expected high precipitation rates, which could result in higher supplementation costs.

4.5.3 Lipid-rich feedstock

The substrates with a high concentration of lipids are usually selected as feedstock for anaerobic digestion due to their high biodegradability and methanogenic potential (Cirne *et al.*, 2007). However, the rapid and easy biodegradability of a lipid-rich feedstock can result in the destabilization of the anaerobic process due to the accumulation of short volatile fatty acids (Chan *et al.*, 2018; Cirne *et al.*, 2007). For this, anaerobic digesters at full scale are sometimes operated at low organic loading rates or with poor concentrations of lipid-rich feedstock (Karlsson *et al.*, 2012). Food industry waste is often lipid-rich feedstock, especially if the process is related to oil or dairy. Example of TE concentration in these feedstocks are shown in the Table 4.3.

The supplementation with trace elements has been studied as a strategy to ensure stable operation and to maximize methane production when lipid-rich feedstocks are treated (Chan *et al.*, 2018; Karlsson *et al.*, 2012). Iron, cobalt and nickel have been described as having high relevance in the degradation of volatile fatty acids due to their role as cofactors in different acetogenesis and methanogenesis enzymes (Karlsson *et al.*, 2012; Pinto-Ibieta *et al.*, 2016). For example, these three trace elements act as a cofactor of the carbon monoxide dehydrogenase, which it is involved in the degradation of acetate in acetogens and methanogens (Karlsson *et al.*, 2012; Ortner *et al.*, 2015). However, other authors have reported that the addition of nickel and cobalt was not enough to prevent the accumulation of fatty acids, supplementation with a more complex

Table 4.3 Trace-element composition of different lipid-rich feedstock.

		S ppt	N ppt	P ppt	K ppt	Al ppt	Cu ppm	Zn ppm	Mg ppm	Fe ppm	Mn ppm	Co ppb	Ni ppb
Vivekanand *et al.* (2018)	Whey	1.00	4.0	8.3	30.5	55.5	8.2	1.75	1600	1950	10	300	2750
Güler and Sanal (2009)	Whey (cow)		0.4	0.68	1.75		0.1	6.57	343	0.27	0.17	130	
	Whey (sheep)		1.2	1.19	1.97		0.17	7.84	477	0.30	0.11	160	
	Whey (goat)		1.1	0.69	2.23		0.13	4.26	334	0.24	0.11	130	
Eftaxias *et al.* (2018)	Emulsified slaughter-house wastes		9.02				0.135	2.30		0.430	0.127	5	8
Zhang *et al.* (2011)	Food waste	0.59	6.4	1.49	0.546	4.31	3.06	8.27	62.5	3.17	0.96	<30	190
Zhu *et al.* (2008)	Food waste				1.112		1.19	2.53	87.7	9.47	0.84		
Zhang *et al.* (2007)	Food waste	2.5	9.7	1.6	2.8	1202	31	76		766	60		2000
Wan *et al.* (2011)	Fat, oil, and grease		1.0	0.03	0.06		579	2498	13.3	29.6	<0.2	<0.2	559
Schmidt *et al.* (2018)	Slaughter-house wastewater							0.49		4.5	0.28	1.89	7.44
Zhang *et al.* (2008)	Palm oil mill effluent	0.40	0.87	0.27	5.53	6299	5.08	6.83	1.065	61.17	8572		
Pinto-Ibieta *et al.* (2016)	Olive mill solid waste	0.39	5.7	0.66			3.82	5.67	1687	224	3.99	16.9	858
Gowda *et al.* (2004)	Coconut cake			1.3			26	56	0.35	516			
Christodoulou *et al.* (2014)	Sunflower cake	0	7.99	3.21	4.87	147				8179			

trace element solution being more effective (Schattauer *et al.*, 2011). Zinc, molybdenum and selenium have also been reported as essential trace elements for the correct anaerobic digestion of slaughterhouse waste (Ortner *et al.*, 2015). In fact, the addition of molybdenum, cobalt and selenium have been successfully used to revert high volatile fatty acid accumulation in anaerobic digesters (Garuti *et al.*, 2018). The supplementation of trace elements for these substrates, especially iron, cobalt and nickel, should compensate for the deficiencies in feedstock composition that could result in the accumulation of volatile fatty acids.

4.5.4 Lignocellulosic waste

Vegetable-waste material is composed of different biomass generated from agricultural and agro-industrial activities. This kind of feedstock has been widely used as anaerobic feedstock, even at full scale (Garuti *et al.*, 2018). However, the mono-digestion of vegetable waste can be a challenge due to its high fibre content, i.e. cellulose, hemicellulose and lignin, as well as a deficiency in trace elements (Cai *et al.*, 2017). The unavailability of trace elements in biogas digesters can be one of the main reason for poor process efficiency in the biomethanization of lignocellulosic material (Demirel & Scherer, 2011). The low concentration of trace elements in many lignocelullosic wastes (Table 4.4) makes necessary their supplementation to maintain biogas production and to avoid destabilization of the process (Demirel & Scherer, 2011; Garuti *et al.*, 2018).

Biomethanization of lignocelullosic waste entails all the stages of the anaerobic digestion, where many enzymes and cofactors are involved requiring different trace elements in each stage. Nickel, cobalt, iron, molybdenum and/or selenium have been reported to enhance biogas production and operational performance in an anaerobic digester treating different green-waste materials. For example, the anaerobic digestion of Napier grass produced 40% more methane after a daily addition of a solution containing nickel, cobalt, molybdenum and selenium (Demirel & Scherer, 2011; Wilkie *et al.*, 1986). However, more recent studies reported that the addition of nickel and cobalt has a limited effect on the anaerobic digestion of lignocellulosic material (Cai *et al.*, 2017). These trace elements are related with the acetogenesis and methanogenesis stages, whereas the hydrolysis has been defined as the rate-limiting step for this type of feedstock (Hendriks & Zeeman, 2009; Ortega *et al.*, 2008). For lignocellulosic waste, supplementation of iron enhanced the biogas production and the biodegradability of the substrate. For example, Khatri *et al.* (2015) reported an improvement in methane production by up to 32% for the anaerobic digestion of maize straw with the addition of 1000 mg/L of Fe. However, Cai *et al.* (2017) reported that the addition of 5 mg Fe/L was enough to improve the anaerobic digestion of rice straw up to 176%. The huge differences in the reported supplementation concentration highlight the necessity of determining the bioavailability of the

Table 4.4 Trace-element composition of different substrates in lignocellulosic waste.

		S ppt	N ppt	P ppt	K ppt	Al ppt	Cu ppm	Zn ppm	Mg ppm	Fe ppm	Mn ppm	Co ppb	Ni ppb
Moraes *et al.* (2015)	Straw	0.76		0.44	7.9	53.8	1.95		580	50.02	11.9	<0.5	430
Serrano *et al.* (2014)	Strawberry waste		2.30	1.24			3.6	<1					11,180
Vivekanand *et al.* (2018)	Cellulose	0.03	1.0	0.0	0.01	2.25	0.0	0.0	0.0	0.0	0.0	10	0.0
Nges *et al.* (2012)	Jerusalem artichoke	0.28	5.34	0.38	1.43		1.24	4.8	1480				230
Wall *et al.* (2014)	Grass silage						5.7	23.5		277	38.4	<250	3600
Wandera *et al.* (2018)	Maize straw		1.5		11.1		7.3	69.7	2316	3372	71	0	54.8
Xi *et al.* (2015)	Wheat straw		4.9				0	65.51	0.83	7470	49.02		4.05
Cai *et al.* (2017)	Rice straw		6.41				0.55	25		294	156	0	5280
Mancini *et al.* (2018)	Rice straw		10.4				15.5	57.6		443		<1.0	2000
Mussoline *et al.* (2014)	Rice straw		5.0	0.91	8.15		15.4	45.8	1325	269		164	3709
Janke *et al.* (2015)	Sugarcane straw	1.61	3.98	0.46	5.00		8.2	7.7	1140	11,460	136	2201	5499
	Sugarcane filter cake	0.05	5.08	0.17	740		12.7	38.1	971	7880	163	971	4133
Li *et al.* (2018)	Grass	3.89	7.94		7.9		12.2	182	1720	168	213	7400	24,500
Wahid *et al.* (2018)	Grass mixture	4.72	4.5	3.81	36.4	274	11.10	32.00	2060	444	112	<300	2740

trace elements, instead of only the total concentration. For the agricultural feedstock, it could be also interesting to determine the fibre composition, since the preponderance of a concrete lignocellulosic compound could require the supplementation of different trace elements. Lignin and cellulose-rich feedstock should be supplemented with trace elements involved in different metalloenzyme-catalyzing hydrolytic reactions, such as manganese (Dismukes, 1996; Romero-Güiza *et al.*, 2016). On the another hand, substrates with a high content of hemicellulose, which can be easily solubilized, should be supplemented with trace elements involved in the acidogenesis and methanogenesis stages (Temudo *et al.*, 2009).

4.6 CONCLUSION

The consideration on the role of TE in a full-scale anaerobic digestion plant is still minimal due to a lack of awareness or knowledge. This chapter highlights key points AD operators and stakeholders should focus on when considering TE in their system. Understanding the complete TE speciation in anaerobic digesters requires time, equipment and expertise, but a simple analysis of operating conditions and feedstock should be enough to give an indication on potential inhibition or deficiency.

The quantification of TEs entering a system (through feedstsock analysis) and the retention potential of the process (reactor type) should allow for estimation of TE quantity in the anaerobic bioreactor. The standard operating condition of the system should evaluate the TE requirement for the microbial community and potential deficiency. Research studies are now developing computer models to afford a better picture of TE speciation/bioavailability based on input parameters (see chapter 5) and give operators an easier platform to assess their own system. Validation and distribution of these models to the AD industry will be a great leap forward in process optimisation as it has been with the publishing of the AD1 model.

Despite great progress in the understanding of TE behaviour and bioavailability, there remains a need to raise awareness of the crucial importance of TE balance in a system. It is the duty of research scientist to be able to cooperate and communicate efficiently with the industry about potential problems and, if possible, offer adequate solutions.

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