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Trace element supplementation as a management tool for anaerobic digester operation: benefits and risks

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Introduction

The energy potential of Anaerobic Digestion (AD) of biowaste should be approximately 200 kWh per tonne of feedstock. However, with only 50-70% organic solid degradation, most AD plants achieve a considerably lower yield. Moreover, long process times (typically, 30 days retention), and the requirements for heating and mixing, increase the costs of energy retrieval and limit the potential advantages of AD applications. Thus, it is important to improve the AD process to overcome constraints and to maximise cost efficiencies.

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 well-working anaerobic digesters. Disruptions to one, or more, of those factors may disturb the activity of specific groups of microorganisms and, thus, impair digester performance.

The requirement for feedstocks well balanced in carbon, nitrogen and phosphorus (C/N/P) is well understood for optimal AD operation. However, **the role(s) of TE has been relatively neglected by researchers and operators**. TE, such as cobalt, nickel or selenium, have 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.

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Improved understanding of the AD process, as well as ongoing technology development, has heightened awareness of the potential of AD biotechnologies for valorisation of sewage sludge, agro-industrial by-products and organic wastes to bioenergy. Process monitoring is fundamental in maintaining optimal AD conditions and in ensuring process stability. This usually involves measurements of key parameters, such as pH, biogas production and temperature.

Optimal conditions for AD plants are achieved when the effective biogas production is close to theoretical production, taking into account the feedstock composition and volumetric throughput. In reality, few anaerobic digesters ever manage to reach this target due to multiple other, interdependent factors limiting digester capacity (Figure 1).

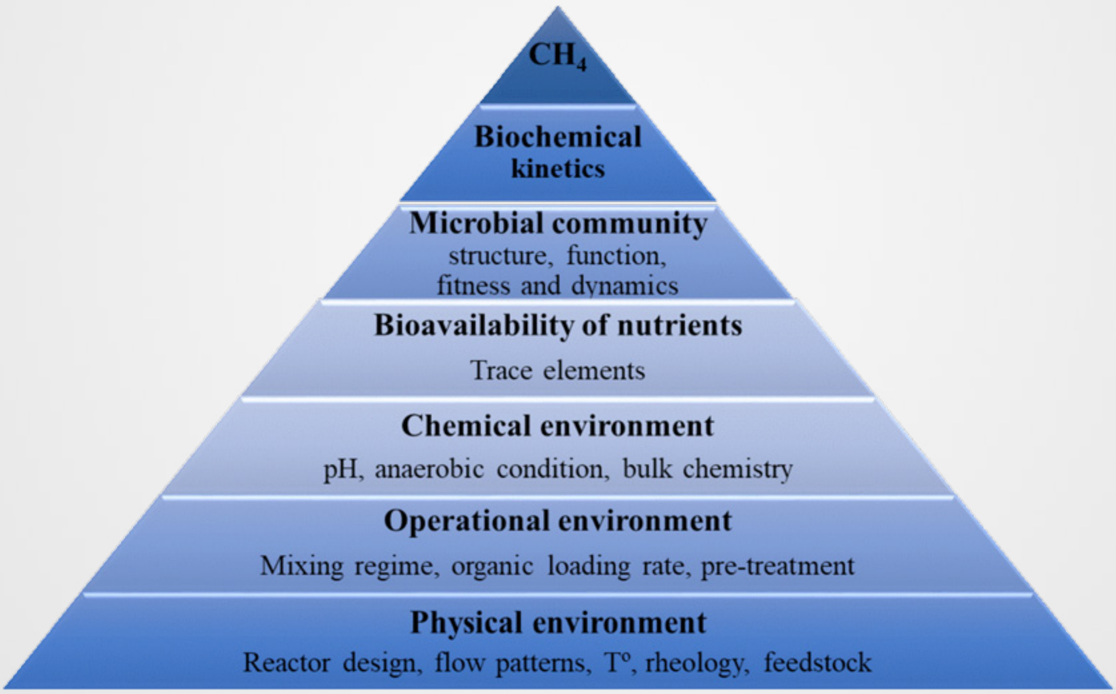

Figure 1. Physico-chemical and biological factors affecting digester capacity.

The bioavailability of nutrients is a key factor, which may influence process performance, since nutrients are necessary for microbial activity. **Deficiency in even only one nutrient may limit microbial activity and, consequently, digester performance** (Figure 2). TE is a term used to include a wide range of micronutrients essential for the microbial community underpinning AD. TE mostly includes elements from the metal groups (e.g. cobalt, nickel, zinc and tungsten) but also other elemental groups, such as metalloids (e.g. selenium).

The key parameters to investigate for potential TE deficiency relate to the volatile fatty acids (VFA) concentrations and pH in digesters. **The accumulation of VFA, such as acetate or propionate, indicates inhibition of methanogenic activity, which may be associated with deficiency in one, or several, TE**. Studies at both laboratory- and industrial-scale have

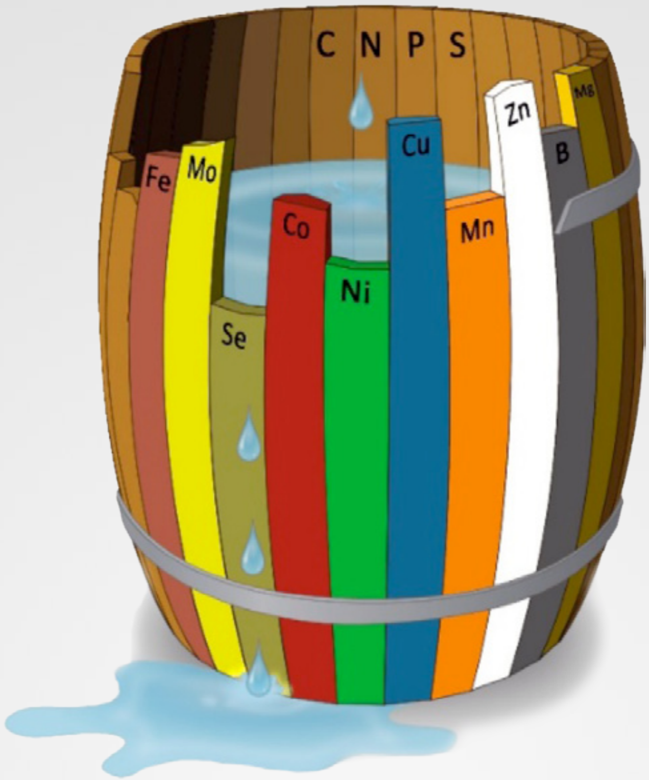

Figure 2: Liebig's law of the minimum illustrating the effect of trace elements deficiency on digester performance

demonstrated that deprivation of elements, such as cobalt or nickel, resulted in impaired substrate conversion to methane, VFA accumulation, and digester acidification and failure (Takashima *et al.* 1990; Osuna *et al.* 2002; Zandvoort *et al.* 2002a; Zandvoort *et al.* 2002b). In fact, in some cases digester performance was recovered simply by supplementing TE and thus stimulating methanogenic activity.

Digester pH is the most accessible and straightforward parameter to monitor VFA accumulation as at high VFA concentrations the pH will fall below 7, potentially reaching a critical point between 5 and 6. With the pH below the 'comfort zone' for methanogenic microorganisms, methanogenesis is inhibited, thus continuing a vicious cycle of further VFA accumulation and leading to AD failure.

Equally, **TE supplementation is also of potential interest when challenging AD operations with increasing rates of organic loading.** In the case of a well-designed, and well-functioning, anaerobic digester reaching the theoretical biogas target, the main question will be whether it could support a higher organic loading rate (OLR), and thus produce more biogas. In this scenario, TE availability may play a critical role in the answer. The Liebig law of minimum teaches that performance will depend on the limiting factor (Figure 2), and that increased loading will upset the biological balance in digesters deficient in an essential element. The result would be impaired performance and the risk of digester failure. Comprehensive analysis is thus highly recommended to determine TE concentrations and to assess TE bioavailability for microorganisms.

Take-away: in case of unexpected VFA accumulation and/or reduced biogas productivity, or to support increased organic loading, digester supplementation with TE should be considered.

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What are the benefits of adding trace elements to a digester?

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The motivation for TE supplementation is to increase the concentration of deficient TE above the limiting level to boost microbial activity, leading to faster feedstock biodegradation. However, predicting **the fate of supplemented TE is complicated as this is governed by several parameters and a variety of complex chemical reactions**. There is a high risk that the supplemented TE will bind strongly with sulfides, or other inorganic and organic ligands, and become non-bioavailable to microorganisms. Therefore, knowledge of the behaviour of supplemented TE in the digester is important in determining the potential for bioavailability.

The first studies on the importance of TE in AD were done in the 1980s, with the work of Callander and Barford (Callander & Barford 1983b, a) and Speece *et al.* (Speece *et al.* 1983; Speece *et al.* 1986). Those studies shifted the view of heavy metals, such as nickel or cobalt, as dangerous substances to essential elements supporting microbial growth in AD. Callander and Barford (1983a,b) also introduced the notion of chemical speciation and availability as fundamental concepts in assessing the role of TE on microbial activities.

In almost 40 years of research on the subject, knowledge on the essential role of TE in AD has expanded significantly but has just scratched the surface of understanding the full chemical and biological dynamics of TE in anaerobic digesters. The research field on TE, and TE supplementation in AD, has been studied by exploring three main topics: TE speciation and TE availability; microbial requirements for TE; and enhancement of biogas production by TE supplementation. *The third topic is the focus of this guide*. Excellent, and comprehensive, reviews have been published on the other two topics to summarise the progress made on the subject (Takashima *et al.* 1990; Zandvoort *et al.* 2006; Demirel & Scherer 2011; Schattauer *et al.* 2011; Thanh *et al.* 2016). Studies confirming the beneficial effect of TE supplementation on biogas production were obtained from batch test and laboratory-scale reactors. The aim has been to quantify the benefits of TE addition and to determine the negative effects of TE deficiency.

Various studies at the laboratory scale have focused on:

- **Digester type, including continuous UASB, multi-stage digesters, etc.**
- **TE supplemented: Cobalt, Iron, Selenium, Nickel, Zinc, etc.**
- **Mode of supplementation: spike(s) or continuous feeding; chemical forms used; etc.**
- **Feedstock type, including municipal wastewater (sewage); food; manures, crops, etc.**

The available research provides a wealth of knowledge of value when assessing individual digester situations. However, it also underscores the complexity of metal supplementation and the specificity of each digester scenario. The various experiments described in the scientific literature were done under controlled, laboratory conditions and using bioreactors of varying sizes from 100 mL to 20 L, and may not be representative of process behaviour in full-scale anaerobic digesters. **In recent years, TE supplementation trials have also been conducted using full-scale AD – in part, due to expanded application of AD in Europe and generally increased awareness of the potential of TE supplementation**.

Lindorfer *et al.* (2012) sampled over 1,500 anaerobic digesters to measure TE compositions and concentrations, and digester performance. They supplemented 60 digesters with TE and observed the impact over a period of four months. Their work demonstrated that, at full-scale, TE supplementation resulted in lower VFA concentrations, more microbial biomass, and enhanced digester performance (Figure 3). They demonstrated improved digester performance within the first few days following TE supplementation, and stable performance for at least three months after TE addition.

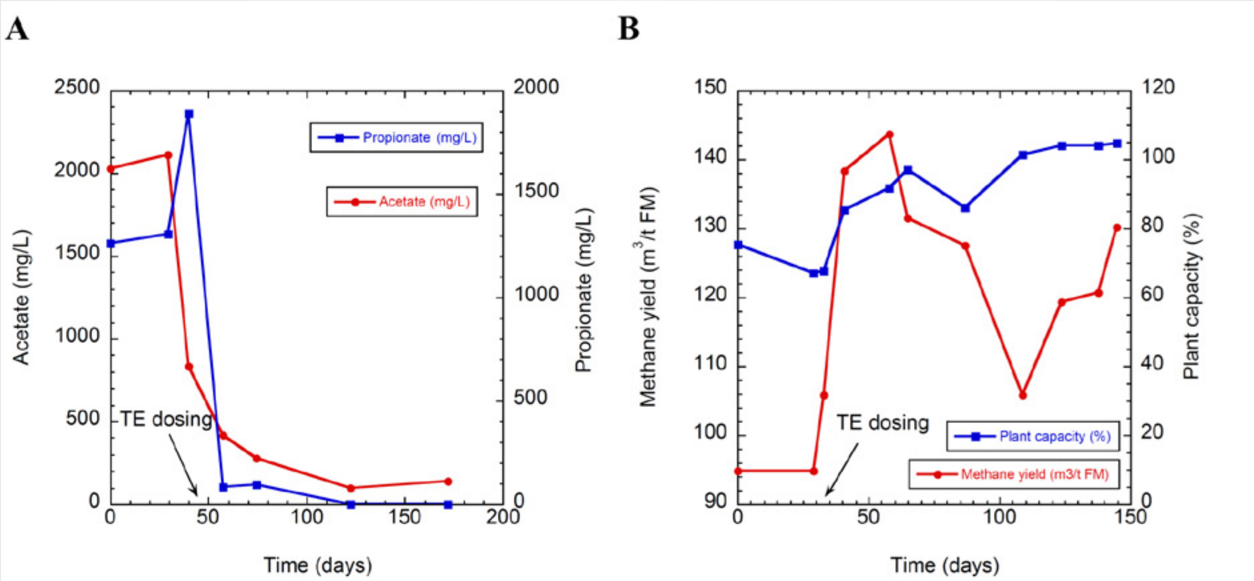

Figure 3. Effect of TE dosing on (**A**) VFA (acetate and propionate) accumulation in a digester working on manure and energy crops (mainly maize), and (**B**) methane yield and plant capacity in a digester working on energy crops only (Reimlingen. Germany). Reproduced from H. Lindorfer, D. Ramhold and B. Frauz 2012 Water Science & Technology 66(9) 1923-1929, with permission from the copyright holders, IWA Publishing.

Finally, one of the main concerns in considering TE supplementation of anerobic digesters is that the optimum, 'bioavailable' concentration lies between two zones of inhibition (i.e. ***deficiency*** *and* ***toxicity***). Over-dosing of at least one TE may result in toxic inhibition of microbial activity, and this risk should be avoided by developing appropriate dosing strategies.

Take-Away: Improved digester performance due to TE supplementation has been demonstrated at laboratory, pilot and full scale. Reduced VFA accumulation and higher biogas output are the main benefits reported.

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Which trace elements do I need to add to my digester?

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As with the human body or with animals, microbial individuals and communities also require optimal conditions of food (i.e. substrate, or feedstock), vitamins, macronutrients (P, N, K) and TE for efficient growth and metabolic activity. TE are essential to microorganisms at low concentrations. Each TE typically plays a unique, and key, role in specific enzymatic functions and cannot be replaced by another TE (except, to some extent in some cases, by elements of the same group). **A list of the main TE and their roles in microbial activity is shown in Table 1**. As examples, cobalt is used as a 'co-factor' (i.e. as vitamin B12) during the last step of the methylotrophic pathway; nickel is used as a 'co-enzyme' (F430) during methyltransferase, the essential step before the production of methane.

Table 1. Selected TE in enzymes of microbial conversions.

Enzyme	Organism(s)	Metal
Methyltransferase	Methanogens and acetogens	Co (B12)
B12-enzymes	Many organisms	Co (B12)
CO-dehydrogenase	Methanogens/Acetogens	Co, Ni, Fe
Acetyl-CoA synthase	*Moorella thermoacetica*	Fe, Ni, Cu
Tetrachloroethene reductive dehalogenase	*Dehalospirillum multivirans*	Co, Fe
Methyl-CoM-reductase	Methanogens	Ni
Uerase	Several organisms	Ni
Hydrogenase	*Desulfovibrio*	Fe
	Escherichia coli	Ni, Fe
	Facultative anaerobes	Cu, Zn
MMO (free)[1)]	*Methylosinus trichosporium*	Fe
Ammoniummonooxygenase	*Nitrosomonas europaea*	Cu
	SOD aerobes, anaerobes[2)]	Fe, Cu, Zn, Mn
Formiate dehydrogenase	*Methylobacterium*	Mo or W
	E. coli	Mo–Se
Formylmethanofuran-dehydrogenase	*Methanobacterium thermoautotrophicum*	Mo or W[3)]
Aldehyde-oxydoreductase	*Clostridium*	Mo or W
	Methanosarcina barkeri	Mo or V, Fe
Chloroperoxydase	*Curvularia inaequalis*	V
Glycin reductase	*E. coli*	Se

The concentration of each TE required for the welfare of an anaerobic digester is difficult to determine as it depends on the compositions and the metabolic activity of the microbial community in the digester, as well as the chemical speciation (and, thus, the 'bioavailability') of the TE. Determining the total TE concentrations is, of course, a good start in quantifying key TE in a digester, but it is not sufficient to allow a determination of the 'bioavailable fraction'. The behaviour of TE is governed by biochemical reactions and TE may be present as free ions, bound complexes or as precipitates, depending on pH, inorganic ligands, or organic macromolecules. The result of those reactions creates a pseudo-chemical equilibrium defining a speciation for each TE and determines the bioavailable fraction.

The bioavailable fraction is defined as the degree to which elements are available for interaction with microbial community but, in reality, is complicated to measure and quantify (van Hullebusch *et al.* 2016).

Several studies (Hughes & Poole 1991; Hassler *et al.* 2004; Worms *et al.* 2006) demonstrated that free ions were bioavailable but there was also a pool of weakly-bound TE (organic and inorganic TE complexes) that can be dissolved, or released, and taken up by the microorganisms (Figure 4). Thus, the aim of TE supplementation should be to target this bioavailable fraction and ensure that at least part of the added TE is beneficial for microbial activity in the digester – expressed as enhanced methane productivity.

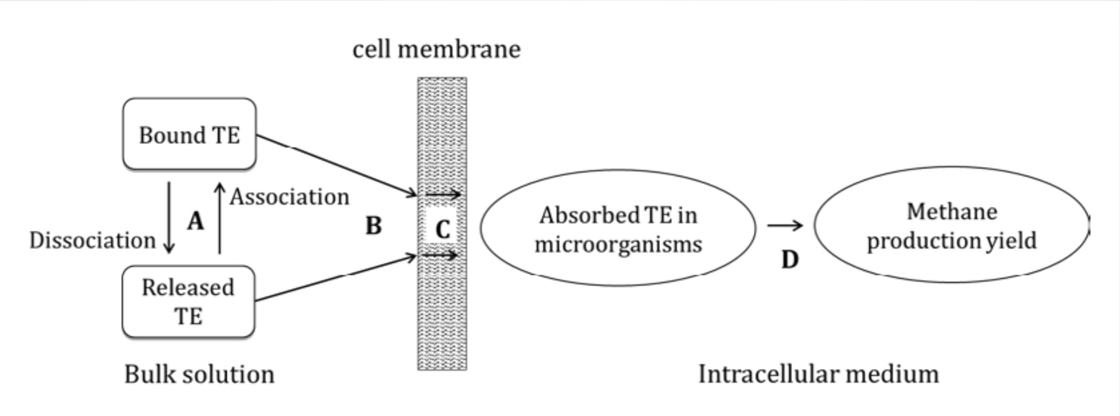

Figure 4: Conceptual simplified representation of TE bioavailability in anaerobic digesters (adapted from NRC (2003)). A, B and C are related to bioavailability processes: TE interactions between phases, transport of TE to microorganisms and bio-uptake of TE through the biological membrane, respectively. D represents the biological response (i.e. methane production yield) as a function of the bioavailable TE intracellular concentration.

Several studies have reported a wide diversity of concentrations of different TE in various anaerobic digesters (Table 2). As with other nutrients, TE are supplemented into the digester along with the feedstock and so the TE concentration will solely depend on the input values. TE concentrations in feedstock are based on a wide range of factors, including the type of material or feedstock, absorption capacity, or even location. Sewage sludge, for example, is composed of a mixture of household, human and industrial wastes, which increases TE diversity and concentrations. Sole agricultural wastestreams, on the other hand, such as maize or wheat, may be severely deficient in one or several TE – especially elements rarely available in soil (Evranos & Demirel 2015). Food waste or food-processing waste are often deficient in iron and selenium (Ariunbaatar *et al.* 2016). Finally, manures, or animal wastes, may present the risk of having very high concentration of specific TE, such as copper or zinc, if animals have been fed with food supplements containing those elements.

Table 2 TE concentration measured or calculated in AD (modified from Schattauer *et al.* (2011).

	References								
	h	d	c	e	f	b	a	g	i
	µM [fresh matter]								
B							0.092 - 1.02 × 10^3		79.5 - 442.1
Ca						>13.5 - 998			
Co	1.02	0.05 - 1.02	8.5 - 339	0.05 - 1.0	0.05 - 1.0	>0.01 - 2.0	1.0	0.4 – 169.7	0.5 - 27.8
Cu							0.9 – 1007.2		0.4 - 25.8
Fe		17.9 - 179	179 - 3.6 × 10^3	17.9 - 179	17.9 - 170	>5 - 902			859 - 1.09 × 10^5
Mg						14.8 × 10^3 - 1.97 × 10^5			2.7 × 10^3 - 1.65 × 10^5
Mn	0.09 - 910						0.09 - 1001		103.2 - 1354
Mo	0.52	0.05 - 0.52	1.04 - 3.64	0.05 - 0.52	0.05 - 0.52	>0.01 - 0.5	0.52	1.7 - 521	1.4 – 4.8
Ni	0.1	0.085 - 8.5	8.5 - 511	0.085 - 8.5	0.085 - 8.5	0.1 – 85.1	0.1	0.4 - 10.6	3.9 - 61.2
S						9.98 – 5.1 × 10^5			1.1 × 10^3 - 7.2 × 10^3
Se (IV)	0.1		1.3 - 4.4	0.1		1 - 10	0.1		0.13 - 5.7
W			0.5 - 1.9	0.5 - 2.2		0.09 - 99.5			
Zn									131 - 1044

a (Sahm 1981), b (Takashima *et al.* 1990), c (Kloss 1986), d (Weiland 2006), e (Seyfried *et al.* 1990), f (Mudrack & Kunst 2003), g (Pobeheim *et al.* 2010), h (Bischofsberger 2005), I (Schattauer *et al.* 2011).

Take-Away: Co, Ni, Fe and Se are usual suspects, and should always be taken into account. However, other essential TE, such as Zn, Cu or W, should also be considered.

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Strategy for trace elements supplementation

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To decide whether TE supplementation of AD is required – and how to approach the dosing process – the decision-support diagram in Figure 5 may be used. The first (green) area considers evaluation of the performance of the digester. **In the case of an underperforming digester and the reason is not clearly found (first steps in Figure 5), then TE limitation might be case.**

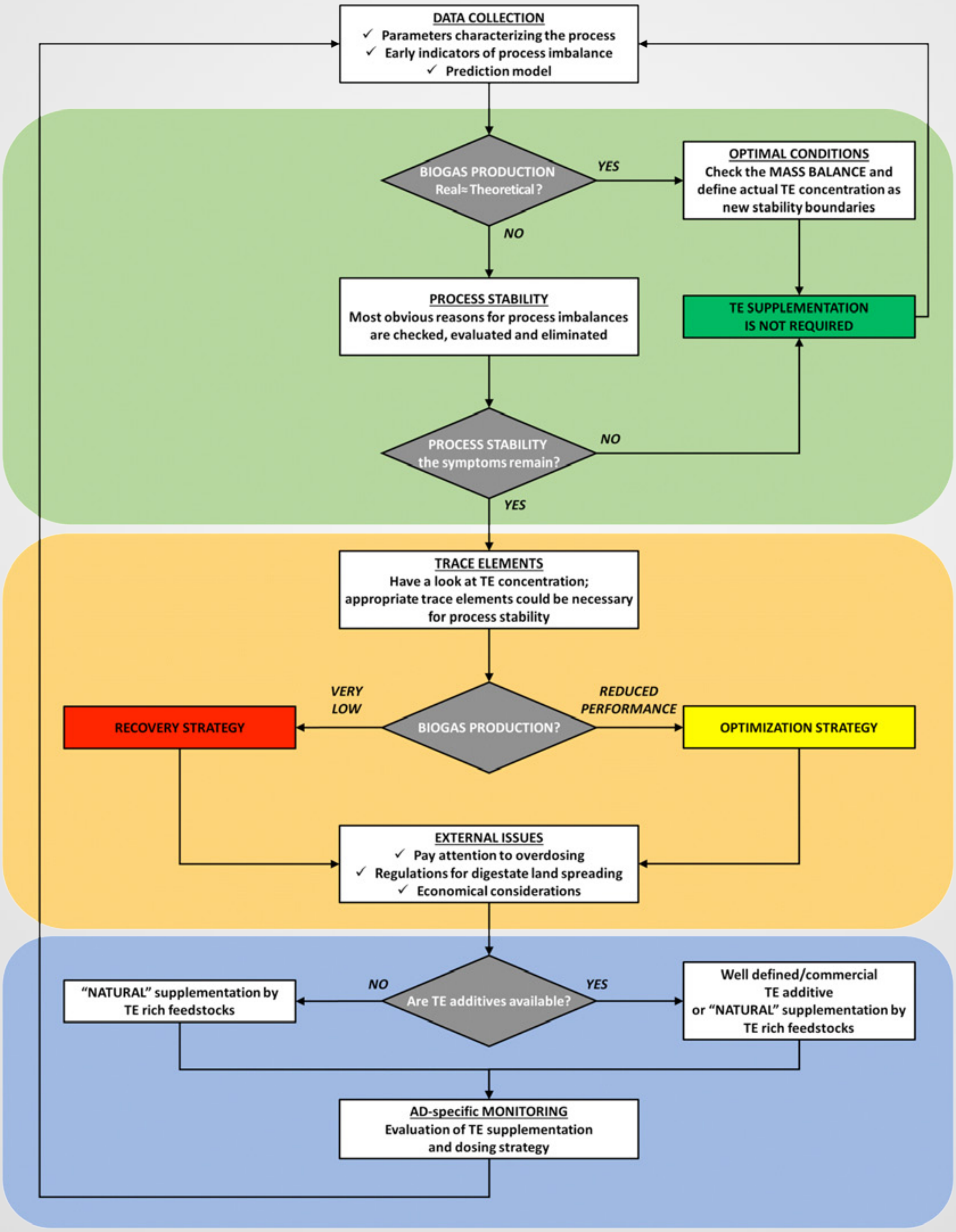

Fig. 5. Following decision diagram for TE management.

At the second area (Yellow Area) a deep study about TE requirements should be done. Extensive elemental analysis of the feedstock to have a global view of AD system is crucial to spot potential deficiency in key TE. Analytical companies often offer this service combined with a maximum methane potential investigation that could be useful to gauge AD performance with its theoretical maximum. Universities and research centres (see below for contact) will also be able to help in the analysis and the interpretation of the results. European Union countries often encourage Industry/Research institute collaboration through funding scheme that will be ideal to support those studies.

Once TE requirements are set, the third are (Blue Area) would take place: how to add them. Once the one or several potential deficient TE has been identified two main directions can be taken to increase their bioavailable concentration. 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 TEs cocktail might quickly recover the AD to normal performance. In a non-urgent case, the work prior starting the TE supplementation should focus on the concentration required, the type of compounds used (salt or organic ligand), and the adding system (continuous or pulse). It is impossible here to give a miracle receipt as each digester is unique and will require a specific attention. However, using literature on previous studies and researcher knowledge, the development of an efficient dosing strategy should be feasible taking into account the type of feedstock, the reactor specificity and the anaerobic digester operating condition.

In the last ten years, many companies with a strong background in vitamin-mineral remix for zootechnical use or mineral fertilizers supply transferred their competences in renewable energies; they created a specific division for biogas sector and they developed a range of products specifically studied to optimize AD. Those additives solutions, containing macro and micro nutrients, can be used to recover anaerobic digesters with TE deficiency or to enhance biogas production. The companies also usually offer a full elemental analysis of the feedstock and laboratory tests to demonstrate the valuable effect of the additive solution on the anaerobic digester performance.

Another option is to use the potential of co-digestion to supplement missing TE if possible. As described above, feedstocks have different content in TE and some might be rich in one or more elements. The use of a co-digestion system may naturally equilibrate the nutrient availability and enhance AD performances (Zhang *et al.* 2011; Romero-Güiza *et al.* 2016). The main advantage of co-digestion is to bring a synergy between two/three compatible feedstocks in term of overall nutrient supplementation (including TE) with a low cost. The mixing of feedstock has also an impact on the speciation of TE with the blending of inorganic fraction and the potential release of bound TE to more available fraction (Tian *et al.* 2015). In both cases, this work should be considered on a long-term basis to first quantify the beneficial effect of TE supplementation on the AD performance. In a second time, the research should focus on the optimisation of the supplementation by understanding the response of the AD to the different stimulation.

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Risks linked with TE supplementation

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Unlike the nutrients, TE introduced in the reactor are not degraded during AD, and their content in the feedstock is highly dependent on anthropogenic sources. Among the main origins of the TE are animal feed additives, food processing industry, flotation sludge, fat residues and domestic sewage.

In order to avoid risks with TE supplementation, it is very important to develop an appropriate dosing strategy, ensuring that each TE concentration is comprised between the two inhibition zones (deficiency and toxicity). Over dosing of at least one TE may result not only in toxicity effects inside the bioreactor, with a decrease in the reactor performance, but also can lead to an environmental risk once the digestate is discharged.

Publicly available specification (PAS) BSI PAS 110:2014 specifies upper limits for Cd, Cr, Cu, Pb, Hg, Ni and Zn in digestate, while for others TE upper safe limiting values are not determined. Several studies have reported lower levels in digestates relative to the standards set by German, British and Spanish legislations (Nkoa 2014), however the long term application of TE and its accumulation in soil over repeated application is not known and needs to be investigated in the future.

COST action ES 1302

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COST action ES 1302: Ecological function of trace metals in anaerobic biotechnologies

The COST action ES 1302 is an action aiming to the establishment of a strong European network on understanding the role of TE to enhance the biogas production. The action started in 2013 and has been running for four years under the chair of Dr. Fernando Fermoso. Five working groups have been created to focus each on a specific research area. The five axis of research are the chemical speciation and bioavailability of TE, the interaction of TE with micro-organisms, the engineering importance of TE in AD industry, the fate of TE in the environment and finally the modelling of TE behaviour in AD.

The network is represented by researcher and industrial from 24 European countries and succeeds to coordinate, enhance and contribute to the research on TE in anaerobic biotechnologies. One of the success of this COST action is to include under a same group a wide network including young and emeriti researchers working on all the aspect of TE roles in anaerobic environment. This network enable researchers to share their knowledge and ensure a continuity between the past, current and future work on this topic.

If you have any queries, ideas or interest in the TE supplementation work please contact the COST ES1302 representatives in your country:

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