Growth media in anaerobic fermentative processes

The underestimated potential of thermophilic fermentation and anaerobic digestion

Hendriks, A. T.W.M.; van Lier, J. B.; de Kreuk, M. K.

DOI

[10.1016/j.biotechadv.2017.08.004](https://doi.org/10.1016/j.biotechadv.2017.08.004)

Publication date

2018

Document Version

Accepted author manuscript

Published in

Biotechnology Advances

Citation (APA)

Hendriks, A. T. W. M., van Lier, J. B., & de Kreuk, M. K. (2018). Growth media in anaerobic fermentative processes: The underestimated potential of thermophilic fermentation and anaerobic digestion. *Biotechnology Advances*, 36(1), 1-13. <https://doi.org/10.1016/j.biotechadv.2017.08.004>

Important note

To cite this publication, please use the final published version (if applicable).
Please check the document version above.

Copyright

Other than for strictly personal use, it is not permitted to download, forward or distribute the text or part of it, without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license such as Creative Commons.

Takedown policy

Please contact us and provide details if you believe this document breaches copyrights.
We will remove access to the work immediately and investigate your claim.

Title

Growth media in anaerobic fermentative processes: the underestimated potential of thermophilic fermentation and anaerobic digestion

A.T.W.M Hendriks*, J.B. van Lier, M.K. de Kreuk

Section Sanitary Engineering, Department of Water Management, Delft University of Technology, Stevinweg 1, 2628CN Delft, The Netherlands

*Corresponding author: A.T.W.M. Hendriks, a.t.w.m.hendriks@tudelft.nl, phone number: +31 6 48758229

Abstract

Fermentation and anaerobic digestion of organic waste and wastewater is broadly studied and applied. Despite widely available results and data for these processes, comparison of the generated results in literature is difficult. Not only due to the used variety of process conditions, but also because of the many different growth media that are used. Composition of growth media can influence biogas production (rates) and lead to process instability during anaerobic digestion. To be able to compare results of the different studies reported, and to ensure nutrient limitation is not influencing observations ascribed to process dynamics and/or reaction kinetics, a standard protocol for creating a defined growth medium for anaerobic digestion and mixed culture fermentation is proposed. This paper explains the role(s) of the different macro- and micronutrients, as well as the choices for a growth medium formulation strategy. In addition, the differences in nutrient requirements between mesophilic and thermophilic systems are discussed as well as the importance of specific trace metals regarding specific conversion routes and the possible supplementary requirement of vitamins. The paper will also give some insight into the bio-availability and toxicity of trace metals. A remarkable finding is that mesophilic and thermophilic enzymes are quite comparable at their optimum temperatures. This has consequences for the trace metal requirements of thermophiles under certain conditions. Under non-limiting conditions, the trace metal requirement of thermophilic systems is about 3 times higher than for mesophilic systems.

Keywords

Nutrient, trace metal, volatile fatty acid, fermentation, anaerobic digestion, mesophilic, thermophilic, enzyme, hydrogen, biogas

Contents

1	Introduction	2
2	Roles of the different macro- and micronutrients.....	3
3	Growth medium formulation strategy	3
3.1	Macronutrients.....	3
3.2	Micronutrients	4
4	Enzyme activity at different temperatures and the influence on the nutrient requirement in thermophilic and mesophilic systems.....	5
5	Specific conversion pathway depends on presence of specific trace metals	7
5.1	Hydrolysis	7
5.2	Acidogenesis	8
5.3	Acetogenesis.....	8
5.4	Methanogenesis	8

43	6	Vitamins	9
44	7	Bio-availability trace metals.....	9
45	8	Toxicity	11
46	9	Typical form of addition of the elements	11
47	10	Conclusion.....	11
48	11	Acknowledgments	12

49

50 1 Introduction

51 Anaerobic digestion or fermentation of organic waste(water) is widely applied and studied. The most
52 commonly pursued anaerobic mixed culture end products are methane (CH₄), hydrogen (H₂) and
53 volatile fatty acids (VFA) (Gujer and Zehnder, 1983; Henze, 2008). Due to the numerous studies on
54 anaerobic digestion and fermentation, results and data are widely available for these processes.
55 However, comparison of the generated results in literature is difficult, not only due to the used variety
56 of process conditions, but also because of the different growth media that are used (Angelidaki et al.,
57 2009; Cheong et al., 2007; Fang and Liu, 2002; Hawkes et al., 2007; Pobeheim et al., 2010; Temudo et
58 al., 2007). Different nutrient media can highly influence research results and so, ideally, the
59 composition of nutrient media should be the same in order to compare the different experiments
60 reported in literature (Bourriaud et al., 2005; Fang and Liu, 2002, 2002; Muñoz Sierra et al., 2017;
61 Turton et al., 1983).

62 In general, a growth medium consists of so called macro- and micronutrients, in which the
63 concentration of each component depends on the required quantity. The elements carbon, oxygen,
64 hydrogen, nitrogen, phosphorus, potassium, sulphur, calcium and magnesium are categorized as
65 macronutrients since relatively large quantities are required, particularly, for cellular growth. All other
66 nutrients, such as the trace elements iron, cobalt and nickel, and vitamins, are mainly used for enzyme
67 or co-factor production. They are needed in much lower quantities and are therefore categorized as
68 micronutrients.

69 A shortage of trace metals can result in lower biogas production (rates) and process instability during
70 anaerobic digestion processes (Fermoso et al., 2008; Pobeheim et al., 2010). Speece (1988) suggested,
71 based on a survey of 30 municipal anaerobic sludge digesters, which included conducting activity
72 assays, that 17 of the 30 digesters were trace metal limited with regard to acetate and propionate
73 degradation rates. The rate of methane production from acetate (8 digesters) and propionate (9
74 digesters) was stimulated by addition of the trace metals iron, nickel or cobalt (Speece, 1988).
75 Furthermore, the addition of trace metals like iron, nickel, cobalt, molybdenum, selenium and tungsten
76 have been reported to increase acetate, propionate and H₂/CO₂ conversion and conversion rates (Banks
77 et al., 2012; Boonyakitsombut et al., 2002; Espinosa, 1995; Moestedt et al., 2015; Ortner et al., 2015;
78 Plugge et al., 2009; Speece et al., 1983). The observed stimulation of the anaerobic digestion process
79 by the addition of metals was probably caused by a low amount of readily available trace metals in the
80 inoculum and substrate (Solis et al., 2002; Zandvoort et al., 2006).

81 To compare reported results of the different studies and to make sure nutrient limitation is not
82 influencing observations ascribed to process dynamics and/or reaction kinetics, a standard protocol for
83 creating a defined growth medium for anaerobic mixed culture processes is urgently required. This
84 paper explains the role(s) of the different macro- and micronutrients, as well as the choices for a
85 growth medium formulation strategy. In addition, the differences in nutrient requirements between
86 mesophilic and thermophilic systems are discussed as well as the importance of specific trace metals
87 regarding specific conversion routes and the possible supplementary requirement of vitamins. Finally,
88 the paper provides some information on the bio-availability and toxicity of trace metals. The overall
89 focus in this paper is on systems with trace metal limitation and maximized growth rates.

2 Roles of the different macro- and micronutrients

A growth medium is a medium in which all the necessary elements (or nutrients) for the growth of cells are present. The necessary elements are elements that are used for energy production, growth, and maintaining cell functions by organisms. In tables 1 and 2, these elements are categorized into macronutrients and micronutrients, and a description of each element is provided, including their role(s) in cellular function, anaerobic digestion and fermentation processes.

3 Growth medium formulation strategy

Many studies on methanogenesis, anaerobic digestion (AD) and mixed culture fermentation have been conducted with synthetic wastewaters, to which growth nutrients must be added. Also many industrial wastewaters lack nutrients and require growth nutrients supplementation prior to anaerobic treatment. Growth media should contain both macro- and micronutrients. However, because of the different roles of macro- and micronutrients in growing (mixed) cultures, the design of a macronutrient medium requires a different strategy than a micronutrient medium. These strategies will be explained in the following sections.

3.1 Macronutrients

Macronutrients (C, N, P, K, Na, S, Ca and Mg) are the elements which are mainly used for anabolism. Since substrate loading rate and the biomass yield determine the biomass formation, required quantities depend on these two factors during the anaerobic conversion process. In table 3, the average biomass yields of the different phases in anaerobic fermentation are given for both mesophilic and thermophilic conditions. The specific composition of the required macronutrient medium depends on the elemental cell composition of the growing biomass, which is given in table 4.

If the macronutrient requirements were expressed in mg/g TSS instead of mg/g VSS, an average VSS/TSS ratio of 0.85 was used for recalculation (Metcalf, 2005; Takashima et al., 2011). Furthermore, it is assumed that the biomass composition of acidifiers, acetogens and methanogens, in mesophilic and thermophilic systems, is the same.

The biomass yields in table 3 are maximum yields. Observed yields can be lower at increased solids retention times (SRTs), because of an increase in maintenance energy, cell lysis and possible predation effects (Metcalf and Eddy, 2003).

The required amount of macronutrients can be calculated using the following equation:

$$C_E = COD_{bio-influent} * Yield * E_{Biomass} \quad (\text{eq 1})$$

C_E = Concentration of target element (mgE/l)

$COD_{bio-influent}$ = Biodegradable Chemical Oxygen Demand in the influent (g/l)

Yield = Biomass Yield (g VSS/g COD_{bio})

$E_{Biomass}$ = Element concentration in biomass (mg Element/g VSS)

In order to avoid macronutrient limitations that could influence the fermentation pattern (Carlsson and Griffith, 1974), it is proposed to use the highest theoretical maximum biomass yield, regardless of the applied SRT, when calculating the initial macronutrient dosage required. After reaching steady state in the process, the observed yield could be determined and the composition of the nutrient solution could be changed accordingly, to minimize overdosing with macronutrients.

Two elements in macronutrients should be specially considered: carbon and sodium.

For carbon dosage no value is proposed. This is because, in many cases, carbon originates directly from the dosed and converted substrate, so “additional” supply is not required. However, in some cases, CO_2 is required for product and/or biomass formation, as is the case for e.g. hydrogenotrophic methanogens (Metcalf, 2005). In addition, the acidifier *Selenomonas ruminantium* takes up CO_2 to form products (Linehan et al., 1978; Paynter and Elsdon, 1970) and *Clostridium kluyveri* specifically

needs CO₂ to form biomass (Tomlinson and Barker, 1954; Tomlinson, 1954a, 1954b). In these cases an additional carbon supply could be considered.

Sodium is an element which is mainly used as a counter ion for cellular buffers, electrolytes and DNA, and as a solute for exchange and transport processes and energy generation (Maret and Wedd, 2014). In general, sodium concentrations of 100-200 mg/l are beneficial for mesophilic anaerobes (McCarty, 1964). There are however, bacteria and methanogens, like halophilic methanogens, which require a high(er) concentration of sodium (Jarrell and Kalmokoff, 1988; Maret and Wedd, 2014; Scherer et al., 1983).

3.2 Micronutrients

Micronutrients are, by definition, essential nutrients or trace elements that are required by an organism in minute amounts. The trace elements are mainly used for the production and functioning of enzymes and co-factors. In table 2 an overview is given of which micronutrients are required for which enzymes.

The (initial) composition of a chemically defined medium is usually based on the cellular composition and desired cell concentration of the microbe of interest and therefore similar to the approach to determine the composition of a macronutrients medium (Greasham and Herber, 1997; White et al., 1990; Zhang and Greasham, 1999). However, when calculating the required amounts of trace elements for AD and mixed culture fermentations this is probably not the most optimised approach, since the cellular composition of all different micro-organisms is usually not known and only overall biomass compositions can be determined. Also, the required amount of maintenance energy of the different micro-organisms can vary depending on operational conditions. For example, maintenance energy requirement will be high(er) under extreme pH conditions, at temperatures higher than the optimal growth temperature of the organisms or when high undissociated volatile fatty acid (VFA) concentrations are present (Röling and Van Verseveld, 1997; Russell, 1992). The latter increase in maintenance energy might be caused by the diffusion of undissociated VFA over the cell wall, where after they will dissociate inside the cell because of more neutral pH values inside the cell (Russell, 1992; Russell and Diez-Gonzalez, 1997). This would lead to increased intracellular protons and dissociated VFA concentrations inside the cell. In order to prevent intracellular pH decrease and VFA accumulation, protons and VFA will be actively transported out (Russell, 1992; Russell and Diez-Gonzalez, 1997; Tijhuis et al., 1993), leading to an increased maintenance energy requirement and thus to reduced growth (Russell, 1992; Russell and Diez-Gonzalez, 1997).

However, to our knowledge, the effect on enzyme production under suboptimal growth conditions is unknown. The enzyme production could be either higher or lower compared to production under optimal conditions. Therefore, when determining the required amount of trace elements, it is assumed that the enzyme production under both optimal and suboptimal growth conditions is similar. This implies the same trace element requirement under both optimal and suboptimal growth conditions.

Because of this assumption the required amount of trace elements should be based on the biodegradable COD concentration of the waste stream to be treated and not on the biomass yield.

Consequently, the amount of required trace elements can be estimated using the following equation:

$$C_E = COD_{bio-influent} * E \quad (eq\ 2)$$

C_E = Concentration of Element (µg/l)

COD_{bio-influent} = Biodegradable COD in influent (g/l)

E = required Element amount (µg Element/g COD_{bio})

In table 5 and 6 a summary of the proposed trace element requirements for mesophilic acidogenic and acetogenic/methanogenic systems is provided. Values are given in micrograms of micronutrients per gram COD_{bio} in the system and were determined via a stepwise approach. First, if available, literature data regarding the mixed culture was collected and inventoried. If data regarding mixed cultures was

not available, data regarding specific key-organisms, which are present in many cases, was sourced. If no data for mesophilic acidogens could be found, the average value of the methanogens was used, multiplied by 5, because the trace element requirement of acidogens is higher than for methanogens (comparison of iron, nickel, cobalt and zinc, table 5 and 6), and hereby preventing limitation.

For some trace elements, some special behaviour was found and is highlighted below. In many cases molybdenum (Mo) and tungsten (W) are acting as antagonists for each other (Andreesen and Ljungdahl, 1973; Ljungdahl and Andreesen, 1975; Plugge et al., 2009; Zellner et al., 1987).

Copper is an element which is commonly required by aerobic bacteria and aerobic archaea, but only required by a small number of anaerobic and facultative bacteria, and facultative anaerobic archaea (Ridge et al., 2008). The only information that could be found in literature regarding the copper requirement of these organisms is presented in table 6. This is in contradiction with the findings of Ridge et al. (2008), who couldn't establish a copper requirement for the anaerobic archaea they tested. Further research is needed to obtain information regarding the possible copper requirements of acidifiers and methanogens. Since no information in literature could be found regarding the requirement of copper for acidogens, the same value as for methanogens has been used. For the cobalt requirement of mesophilic methanogens two different values are mentioned in literature. The higher value of 25 µg/g COD_{bio-influent} should only be used in the case of direct conversion of methanol into methane (Florencio et al., 1994).

The order for the different required concentrations of trace elements for methanogens is: Fe>Ni/Co>Mo (and/or) W > Zn>Cu/Mn according to Ferry (2010) and Glass and Orphan (2012). Table 6 follows this order, with the exceptions of zinc and copper.

To our knowledge an order of trace element concentrations for acidifiers doesn't exist.

In case of biomass retention in an anaerobic reactor (SRT>HRT), intracellular enzymes also retain in the reactor, implying that the trace metal requirement for intracellular enzymes decreases. Extracellular enzymes, however, can be bound to the (particulate) substrate or free moving in the liquid. In case the substrate is also retained in the reactor, the trace metal requirement would decrease, but if the substrate is washed out due to the low HRT, the trace metal requirement is determined by this HRT and will not change due to biomass retention.

4 Enzyme activity at different temperatures and its influence on the nutrient requirement in thermophilic and mesophilic systems

Different operational temperatures are used in anaerobic fermentation and digestion systems; optimal mesophilic conditions vary from 30 to 40°C, while moderate thermophilic conditions have an optimal operational temperature range of 50 to 60°C (Madigan et al., 1997; Metcalf, 2005). A frequently cited advantage of thermophilic systems is the possible higher conversion rate, or higher loading rate that can be applied, in comparison to mesophilic systems (Ahn, 2000). The higher conversion rates could be caused by higher growth rates; thermophilic methanogens have a growth rate which is, in general, a factor of 2 to 3 times higher when compared to mesophilic homologues methanogens (Borja et al., 1995; Lier, 1995; Speece, 1996). Taking into account that the maintenance energy requirement of thermophilic bacteria and archaea at their optimal growth temperature is higher than the maintenance energy requirement of mesophilic bacteria and archaea at their optimal temperature (Borja et al., 1995; Lier, 1995), then the substrate conversion rates in thermophilic systems could possibly increase even more than the increase of the growth rate. For example, Cecchi et al. (1991) mentioned that the first order kinetic constant of substrate utilization of municipal solid waste is four times larger under thermophilic conditions than under mesophilic conditions. The higher conversion rates of particulate substrates under thermophilic conditions could however, also be caused by other factors; like increased access to the substrate, higher solubility of the substrate, lower liquid viscosity (better mixing) or higher diffusion rates of soluble compounds.

One would expect that at higher temperatures the specific conversion rates of enzymes would also be higher. However, the specific rates of thermophilic enzymes and their mesophilic homologue counterparts are often described as being similar at their respective temperature optima (Amelunxen and Murdock, 1978; Danson et al., 1996; Fágáin, 1995; Huber and Bennett, 1983; Varley and Pain, 1991; Wolf-Watz et al., 2004; Zavodszky et al., 1998). Furthermore, the catalytic efficiencies of thermophilic, mesophilic, and psychrophilic enzymes appear to be similar at their respective operational temperatures (Coquelle et al., 2007; Georlette et al., 2003). Enzyme activity is found to be dependent on enzyme conformational flexibility (Artymiuk et al., 1979; D'Auria et al., 1999; Frauenfelder et al., 1979; Huber and Bennett, 1983; Lipscomb, 1970). Conformational flexibility is the ease with which the shape of an enzyme can be altered. Usually it is a modification to the tertiary structure of an enzyme as a consequence of changes in pH, temperature, ionic strength of the environment, or the binding of a substrate to an enzyme (Huber, 1979). This conformational flexibility of mesophilic and thermophilic enzymes is found to be similar at their respective temperature optima (Daniel et al., 1996; Jaenicke, 1996; Zavodszky et al., 1998). Furthermore, Feller (2010) observed from crystal structures of extremophilic enzymes that all reactive side chains as well as most side chains pointing towards the catalytic cavity are strictly conserved, compared to mesophilic enzymes. Crystal structures of enzymes are the structures of an enzyme at the atomic level in different conformational positions (Huber, 1979). The three-dimensional structures of mesophilic and thermophilic enzymes appear to be superposable (Auerbach et al., 1998; Chi et al., 1999; Hopfner et al., 1999; Isupov et al., 1999; Maes et al., 1999; Russell et al., 1997; Tahirov et al., 1998). This all suggests that the overall catalytic mechanism, reaction pathway and enzymatic properties of the relevant different enzymes are similar under mesophilic and thermophilic temperature conditions (Bauer and Kelly, 1998; Jaenicke, 1991; Ljungdahl, 1979; Vieille et al., 1995; Wrba et al., 1990; Zwickl et al., 1990).

In addition to having equivalent conversion rates and catalytic routes, thermophilic enzymes also (roughly) double their rate with every 10°C increase (Q_{10} of 2) just like mesophilic enzymes (Elias et al., 2014). This underlines the impression that mesophilic and thermophilic enzymes are fully comparable at their optimum temperatures; regarding structure, catalytic route and thermo-sensitivity. This would imply that the increased conversion rates found in thermophilic systems, compared to mesophilic systems, is not due to increased conversion rate per enzyme but is possibly caused by a higher concentration of enzymes in these systems. Very recently, Ghasimi et al. (2015) found strong indications of higher enzyme concentrations in thermophilic systems in comparison to mesophilic systems. Further research is needed to confirm this.

If the enzyme concentration indeed is a factor of 2 to 3 times higher in a thermophilic system compared to a mesophilic system, the amount of micronutrients that are needed for the good functioning of a thermophilic system should also be 2 to 3 times higher compared to a similar mesophilic system. This reasoning is in line with several research results. For example, Takashima et al. (2011) investigated the iron, nickel, zinc and cobalt requirements of a mesophilic and thermophilic system during the conversion of glucose to methane and found that the requirements for the thermophilic system were 2.2 to 7.8 times higher than those for the mesophilic system. Uemura (2010) also concluded that thermophilic anaerobic digestion has a higher trace element requirement than mesophilic anaerobic digestion. Zellner and Winter (1987) investigated the tungsten requirements for hydrogenotrophic methanogens and the results showed that the thermophilic methanogens had a tungsten requirement that was at least 2.5 times higher than the tungsten requirements of the mesophilic methanogens. *Clostridium Pastuerianum* and *Clostridium Welchii* need respectively 0.11 (Schönheit et al., 1979a) and 0.12-0.16 mg Fe/g COD_{substrate} (Pappenheimer and Shaskan, 1944) for optimal growth, which is in line with the amount that Takashima et al. (2011) found for the mesophilic acidogenic phase of digestion (0.177 mg Fe/g COD_{substrate}). According to Nishio et al. (1991) the optimum iron dosage for the thermophilic *Clostridium Thermoaceticum* is 0.28-0.84 mg Fe/g COD_{substrate}, which is about 2.5-5 times higher compared to mesophilic Clostridia. This is also in line with the results of Takashima et al. (2011), who found an iron demand for thermophilic systems that was 2.25 times higher than for mesophilic systems.

From the preceding information it can be concluded that mesophilic and thermophilic enzymes are similar in catalytic route, structure and enzymatic activity and that the trace element requirements for both mesophilic and thermophilic systems will increase if sludge loading rates (kg COD/kg VSS.day) increase (according to equation 2).

The proposed required amount of trace elements for thermophilic acidogenic and acetogenic/methanogenic systems are provided in tables 7 and 8. Values are given in microgram of micronutrient per gram biodegradable COD in the system. These values were determined following the same stepwise approach as for the mesophiles. If no data for thermophilic acidogens or methanogens could be found, the proposed amount for mesophilic acidogens or methanogens was used, multiplied by a factor of 3. For copper the same value as for the mesophilic systems was used without multiplication, because copper is very toxic (Lin and Chen, 1997; Sanchez et al., 1996).

Very little is known regarding the boron requirements for the growth of anaerobic bacteria and for Archaea these requirements have yet to be evaluated (Kabay et al., 2015). The growth of *Saccharomyces cerevisiae* is however, stimulated by boron (Bennett et al., 1999). Boron is in some cases also used for bacterial quorum-sensing (Chen et al., 2002). The addition of boron to the nutrient solution is by the experimenter's discretion.

5 Specific conversion pathways depend on the presence of specific trace metals

The proposed approach (above) to define the micronutrient solution is very general. It should be noted that different fermentation pathways may require a different composition of trace elements in solution. The section below describes some deviations from the general approach. Likely, the overview is far from complete, since many possible fermentation routes and their specific trace element requirements have not been reported in literature or are not yet known. If the specific described route is the dominant or preferred route, the required trace elements should be present in sufficient bio-available quantities. This means they should either be in solution, dissolving at a non-limiting rate, or be liberated, in sufficient quantities, during bio-degradation of the substrate.

The AD process can be interpreted as a sequence of four different microbial conversion steps; hydrolysis, acidogenesis, acetogenesis and finally methanogenesis (Metcalf, 2005). The specific conversion pathways in each of these steps depends on the presence of specific trace elements as illustrated below.

5.1 Hydrolysis

Hydrolysis is the process in which exo-enzymes and/or membrane bound enzymes convert complex particulate compounds into less complex dissolved compounds. The most important hydrolytic enzymes are cellulases, amylases, proteases and lipases and are produced by acidogenic bacteria. The working principle of these enzymes is discussed in depth in several papers (Brahmachari et al., 2017; Rao et al., 1998; Schwarz, 2001). The functioning of some of these hydrolytic enzymes also depends on trace metals. For cellulases this is calcium, which is amongst others, important for the folding of the cellulases (Brahmachari et al., 2017; Lytle et al., 2000; Schwarz, 2001). Amylases need in many cases calcium for their activity, structural integrity and stability (Brahmachari et al., 2017). For some proteases zinc, cobalt, manganese and/or calcium are essential, whereby the first three can stimulate the activity of amylases (Bertini and Luchinat, 1994; Holmes and Matthews, 1981; Latt et al., 1969; Jisha et al., 2013; Rao et al., 1998). Lipases generally don't require metals to function, but the lipase activity is in many cases stimulated by calcium (Gupta et al., 2004).

To our knowledge there is no information present regarding the amount of produced hydrolytic enzymes by different bacteria during mixed culture fermentation or digestion, and thus the trace metals requirement regarding hydrolytic enzyme production is also difficult to estimate: further research on this topic is needed.

5.2 Acidogenesis

Acidogenesis is the step in which organic compounds are mainly converted into VFAs during microbiological processes. The conversion of organic compounds into acetate and butyrate is accompanied by hydrogen formation. This involves hydrogenases and therefore the trace elements iron, nickel, zinc, and selenium are important (see table 2).

In some cases, such as for some acetate-propionate producing *Selenomonas* strains, there is no production of hydrogen during fermentation (Scheifinger et al., 1975). Nonetheless, hydrogenases are involved in the production of propionate (Henderson, 1980). This means that the trace elements iron, nickel, zinc and selenium are still important. In addition to these elements, in some cases vitamin B₁₂ is also required for the production of propionate (Chen and Wolin, 1981; 1992). Vitamin B₁₂ is a cobalt containing vitamin. The bacteria *Selenomonas ruminantium* and *Megasphaera elsdenii* can produce vitamin B₁₂ independently and therefore, only need the supplementation of cobalt (Dryden et al., 1962a). Bacterium *Prevotella ruminicola*, however, is not capable of producing (enough) vitamin B₁₂ for itself (Chen and Wolin, 1981). In this case, the vitamin needs to be added or produced by other bacteria, e.g. *Selenomonas ruminantium*, in the culture. It has been observed that when insufficient vitamin B₁₂ is present succinate is produced instead of propionate (Chen and Wolin, 1981; Strobel, 1992). Also other acidifiers have a vitamin or amino-acid requirement like several cellulolytic acidogens (Scott and Dehority, 1965) and *Megasphaera elsdenii* (Miura et al., 1980). An overview of several acidifiers having a vitamin or amino-acid requirement can be found in Hobson and Stewart (1997).

5.3 Acetogenesis

Acetogenesis comprises of microbiological processes where the products of fermentative bacteria are converted into acetate, hydrogen and carbon dioxide. To our knowledge only (part of) the trace element requirement of the syntrophic propionate oxidizer *Syntrophobacter fumaroxidans* is known, which requires iron, selenium and tungsten to produce formate dehydrogenases (de Bok et al., 2003), while analysis of other trace elements needed for acetogenesis is lacking: Therefore, further research is needed to obtain a better understanding of their trace metal requirements.

5.4 Methanogenesis

Methane can be formed via three different pathways; namely the hydrogenotrophic, methylotrophic and aceticlastic pathway (Glass and Orphan, 2012). The different pathways are explained below.

Hydrogenotrophic pathway

The formation of methane from H₂/CO₂ and formate is called hydrogenotrophic methanogenesis. Important trace elements for the conversion of H₂/CO₂ and formate to methane are iron, nickel, cobalt, selenium, molybdenum and tungsten (Banks et al., 2012; Espinosa, 1995; Jiang, 2006; Kim et al., 2002; Osuna et al., 2003; Öztürk, 1991; Plugge et al., 2009; Worm et al., 2009). This hydrogenotrophic pathway is sometimes found to be the dominant methane formation pathway, for example, in digesters with high ammonium concentrations (Angelidaki and Ahring, 1994; Demirel and Scherer, 2008; Gallert et al., 1998; Koster and Lettinga, 1984; Zinder, 1990).

Methylotrophic pathway

The direct formation of methane from methanol is called the methylotrophic pathway. Important trace elements for the conversion of methanol to methane are iron, zinc, nickel and especially cobalt (Fermoso et al., 2008; Florencio et al., 1994; Nishio et al., 1992; Zandvoort et al., 2002a; Zandvoort et al., 2002b; Zandvoort et al., 2004). For the direct conversion of methanol to methane the cobalt concentration should be in the range of 0.5-2 µM. However, at higher cobalt concentrations methanol degradation may proceed via acetate formation and subsequent aceticlastic methanogenesis, or via oxidation to CO₂ and H₂ followed by hydrogenotrophic methanogenesis, given that the H₂ partial pressure is low enough (Florencio et al., 1994; Jiang, 2006)

Aceticlastic pathway

The formation of methane from acetate by splitting the acetate molecule is called the aceticlastic pathway. Important trace elements for this conversion are iron, nickel, cobalt and zinc (Boonyakitsombut et al., 2002). In some cases selenium has a positive influence on the acetate conversion to methane (Banks et al., 2012). Acetate, however, can also be anaerobically oxidised to H_2/CO_2 and then to methane (Cord-Ruwisch et al., 1988). In that case, the trace elements essential for the hydrogenotrophic pathway should be present in sufficient bio-available quantities. Unfortunately, the required trace elements for acetate oxidation are still unknown.

6 Vitamins

Many methanogenic archaea need vitamins if they are mono-cultured or cultured without acidifiers (Jarrell and Kalmokoff, 1988; Speece and McCarty, 1964; Wolin et al., 1963). Also some (cellulolytic) acidifiers and lactic bacteria require vitamins for growth (Bornstein and Barker, 1948; Mulder, 1990; Pritchard and Coolbear, 1993). However, it has been shown that bacteria can synthesize certain vitamins (Bechdel et al., 1928; Dryden et al., 1962b; Hill, 1997; LeBlanc et al., 2013; Scott and Dehority, 1965; Ye et al., 1996). Mixed culture fermentations often do not need additional vitamin supplementation to function. There is apparently a(n) (inter)dependency of bacteria and archaea regarding vitamins. The latter was already hypothesized by Thompson (1942).

Most anaerobic mixed cultures are self-sustaining with regard to vitamins and amino acids and a simple medium is sufficient (Mulder, 1990; Speece and McCarty, 1964). However, in some cases, e.g. when treating specific industrial wastewaters or in pure culture studies, the production of sufficient vitamins to sustain the biological processes are lacking (Bornstein and Barker, 1948; Jarrell and Kalmokoff, 1988; McCarty and Vath, 1962), although the specific required vitamins and/or amino-acids are often unknown in these processes. In these cases the use of yeast extract (YE) is advised, since this provides a cocktail of the necessary vitamins, amino-acids and trace elements for bacteria and methanogens (Gonzalez-Gil et al., 2003). It is proposed to use a YE addition of 0.05 gYE/gCOD_{bio-influent}. This can be specifically fine-tuned for each case. E.g. in cases where the requirements of the organism are known, it is advised to use the defined medium generally used to culture this specific organism. For example, lactic acid bacteria have exact nutritional requirements. The yeast extract (YE) requirement is about 0.15 gYE/gCOD_{bio-influent} for lactic acid production without cell recycle, but only 0.04 gYE/gCOD_{bio-influent} with cell recycle (Oh et al., 2003; Pritchard and Coolbear, 1993). *Clostridium kluyveri* achieves maximum growth rate at a YE concentration of about 0.5 gYE/l, if acetate and ethanol are not limiting (Bornstein and Barker, 1948). In these cases the yeast extract dosing should be adjusted to the organism aimed for.

7 Bio-availability of trace elements

Trace element bio-availability in fermentation systems can be low because of the formation of precipitates. The solubility of trace metals is, in many cases, mainly determined by the concentrations of sulphide, carbonate and phosphate and to a lesser extent by hydroxide (Callander and Barford, 1983a). Making a nutrient solution by mixing various compounds can lead to the loss of bio-availability of trace elements because they form new salts with lower aqueous solubility compared to the original constituent chemical compounds. This possibly leads to undesired precipitation of the elements (Allwood and Kearney, 1998; Gonzalez-Gil et al., 1999). Care should be taken when combining nutrients like trace elements and phosphates in concentrated media, which can form precipitates. Addition of chelating agents results in large reductions in the free element ion concentrations, but can still keep them available for the microbial population (Callander and Barford, 1983a, 1983b). If the chelator concentration and stability constants are high enough, the free element ion concentration can be reduced to concentrations below the solubility products of sulphide, carbonate and phosphate, thereby preventing or inhibiting precipitation. Chelating agents also reduce the potential toxicity of soluble trace elements by chelating them (Agrawal et al., 2011; Milanovich et al., 1975). The most frequently used chelators are citric acid, Nitrilotriacetic acid (NTA) and

Ethylenediaminetetraacetic acid (EDTA), which are discussed separately below. Care must be taken when selecting which chelating agent to use because these agents can make trace elements more or less bio-available depending on the conditions. If the microorganism has an element binding agent that is stronger than the chelating agent, the element will be more bio-available, if not, it will be less bio-available. Callander and Barford (1983) proposed a method to calculate the soluble elements' ion concentrations. This proposed method works for defined media. However, with a complex medium (which contains (undefined) natural chelating compounds) there can be a significant discrepancy between the calculated level of free element ions and the measured amount of free element ions (Callander and Barford, 1983b). Therefore, it seems that organic ligands from microbial or vegetable origins; such as amino-acids, polypeptides and humic acids, probably play a major role in the bio-availability of trace elements (Callander and Barford, 1983b; Sillén et al., 1964; Speece, 1996). It is possible that over time an anaerobic process becomes able to generate, in sufficient amounts, the necessary chelators that make trace elements bio-available (Speece, 1996). Chelators produced by in situ microorganisms could be dependent on the specific sludge load and type of substrate that is used. Additional trace element supplementation has been shown to be beneficial when changing the substrate or during non-steady state conditions; such as a start-up, change in loading rate, or change of substrate (Henry et al., 1996). At higher temperatures the solubility product constant of carbonate and phosphate metal salts is in many cases lower (Braun, 1991; Friedfeld et al., 1998). Therefore, freely available metal concentrations are generally lower in thermophilic fermentation or anaerobic digestion systems compared to mesophilic systems, increasing the importance of (natural) chelators at elevated temperatures.

As mentioned above, the most commonly used chelators are citric acid, NTA and EDTA. These chelators require at least two of the carboxylic acid groups to be dissociated before they can act as chelators (Rivers and Umney, 2003). For this reason, the different pKa values of citric acid, NTA and EDTA are provided in table 9.

Each chelator has its advantages and disadvantages. An overview of the advantages and disadvantages of these three chelators is given in the following sections.

Citric acid

Citric acid is a chelator which is very soluble (Dalman, 1937). To guarantee two dissociated carboxylic acid groups, the pH must be above 4.76, according to table 9. However, a great drawback of the use of citric acid, is that it can be (an)aerobically degraded (Aquino and Stuckey, 2007; Veeken, 1999).

NTA

Nitrilotriacetic acid (NTA) is a chelator that is hardly soluble in its acidic form. To increase the solubility of NTA and to let NTA chelate trace elements, the pH has to be increased to 6 or higher (Rivers and Umney, 2003). However, the pH shouldn't be too high because then the trace elements could precipitate as hydroxides over time (Rivers and Umney, 2003). Free NTA does not interfere with anaerobic digestion (Aquino and Stuckey, 2007). Although NTA is quite resistant to biodegradation it can be degraded given enough time (Nörtemann, 2005).

EDTA

EDTA increases the bio-availability of trace elements. Addition of EDTA causes a reduction in the amount of trace elements that needs to be added (Vintiloiu et al., 2013) and also does not affect the activity of enzymes (Mahadevan et al., 1977). A drawback is that free EDTA can reduce the methanogenic production rate. It is suggested that this is because EDTA can act as a stronger chelator than the chelating sites on the cell surface (Aquino and Stuckey, 2007). EDTA can also chelate bivalent cations like Ca^{2+} and Mg^{2+} which could possibly result in the reduction of biofilm and granule stability (Grotenhuis et al., 1991). Therefore, care should be taken to ensure that most EDTA is in its chelated form.

Because of the degradability of citric acid, and the possibility of excessive chelation of trace elements and bivalent cations by EDTA, NTA is the preferred chelator to use in our opinion.

8 Toxicity

Trace elements are necessary, but at high concentrations they can become inhibitory or even toxic. (Nandan et al., 1990). Inhibition and/or toxicity is often caused by the chemical binding of trace elements to enzymes, resulting in disruption of the enzyme structure and consequently, inactivation of the enzyme itself (Vallee and Ulmer, 1972). The toxicity of trace elements depends on their chemical forms and their dissolved concentrations (Lin, 1993). Trace element toxicity can be reduced or prevented by chelation (Aquino and Stuckey, 2007; Babich and Stotzky, 1983). In flocculated or granular form, methanogens in anaerobic sludge show higher resistance to trace element toxicity in comparison to single cell cultures (Lin and Chen, 1997; Pedersen and Sayler, 1981). However, adaptation by the biomass culture to higher trace element concentrations can also occur over time (Chen et al., 2008). This may be the result of internal changes in the predominant species, or of a shift in the population (Chen et al., 2008).

Of the microorganisms involved in the anaerobic digestion process, methanogens are generally considered to be the most sensitive members of the anaerobic consortia to trace element toxicity (Feng et al., 2010). Acidogens are thought to be more resistant than methanogens (Zayed and Winter, 2000). In tables 10 and 11 an overview is provided of the literature resourced inhibitory concentrations for different trace elements for mesophilic acidogens and mesophilic methanogens. The inhibitory concentrations of trace elements for thermophilic systems is not presented due to a lack of information. For practical purposes, the inhibitory/toxic trace element concentration values for mesophilic systems can also be used for thermophilic systems.

For both mesophilic acidogens and methanogens the trace element concentration at which inhibition started, as well as the IC_{50} concentrations, were sourced from the literature. The 'concentration at which inhibition started' means the concentration at which a reduction of maximum growth rate starts to occur.

For iron, cobalt, zinc, manganese, molybdenum, selenium, tungsten and boron no concentrations at which inhibition started, and IC_{50} concentrations for mesophilic acetogenic and methanogenic (suspended cell) cultures could be found.

If no information is present regarding the toxicity of a trace element, the value of the most inhibitory element can be used for that specific group. References indicate that the most inhibitory element for acidogens is nickel and for methanogens it is copper (Lin and Chen, 1997; Sanchez et al., 1996).

9 Typical form of addition for the elements

In table 12 an overview is given of the typical forms that are commonly used to supply the required macronutrients and trace elements.

Bacteria and Archaea require elemental sulphur. If sulphate is dosed it will be reduced to sulphide at the expense of available COD. Most organisms can use sulphide or cysteine and/or methionine to fulfil this requirement (Jarrell and Kalmokoff, 1988). However, sources of sulphur other than sulphide can result in lower growth rates (Daniels et al., 1984). Excess sulphide has however, amongst others, the disadvantage of promoting formation of H_2S or sulphide precipitates, depending on the pH. The sulphide precipitates can cause issues such as fouling of electrode surfaces. Overdosing of sulphide should thus be avoided if possible.

10 Conclusion

Until now most published results on anaerobic digestion and fermentative conversions are not comparable because of the different nutrient media used. This review paper is an attempt to standardize the composition of nutrient media for growing anaerobic consortia. After an extensive review of current literature, it can be concluded that the required amount of macronutrients should be based on the biomass growth, while the required amount of trace metals should be based on the biodegradable COD concentration in the influent. A distinction can be made regarding nutrient requirements between anaerobic digestion and fermentative processes without the aceticlastic/methanogenic phase. It should be noted that thermophilic bacteria and archaea have a trace metal requirement, which is possibly a factor of 2 to 5 times higher than for mesophilic bacteria and archaea.

For stable operation of an anaerobic digester, an analysis should be made of the trace metals present in the organic waste(water) stream that will be treated to judge if additional trace metal dosage is required. If the substrate of a stable running digester is changed, the present microbial population has to adapt to this new substrate. During the adaptation period it is advised to add trace metals and vitamins during this adaptation period, where after this dosage could be slowly reduced. The same applies for fermentation, although addition of vitamins and amino-acids could lead to a change in fermentation product spectrum.

Acknowledgments

The financial support from the Dutch Technology Foundation STW, which is part of the Netherlands Organisation for Scientific Research (NWO), and which is partly funded by the Ministry of Economic Affairs and the company Paques B.V. (project nr. STW-Paques 12998) is gratefully acknowledged. Furthermore, Mostafa Zahmatkesh is gratefully acknowledged for his time and input during fruitful discussions.

Conflict of Interest statement

The authors report no conflict of interest.

References

- Adams, M.W.W., Jin, S.-L.C., Chen, J.-S., Mortenson, L.E., 1986. The redox properties and activation of the F420)-non-reactive hydrogenase of *Methanobacterium formicicum*. *Biochim. Biophys. Acta BBA - Protein Struct. Mol. Enzymol.* 869, 37–47. doi:10.1016/0167-4838(86)90307-9
- Agrawal, J., Sherameti, I., Varma, A., 2011. Detoxification of heavy metals: state of art, in: *Detoxification of Heavy Metals*. Springer, pp. 1–34.
- Ahn, J., 2000. A comparison of mesophilic and thermophilic anaerobic upflow filters. *Bioresour. Technol.* 73, 201–205. doi:10.1016/S0960-8524(99)00177-7
- Ahring, B.K., Westermann, P., 1985a. Methanogenesis from acetate: Physiology of a thermophilic, acetate-utilizing methanogenic bacterium. *FEMS Microbiol. Lett.* 28, 15–19. doi:10.1111/j.1574-6968.1985.tb00755.x
- Ahring, B.K., Westermann, P., 1985b. Sensitivity of thermophilic methanogenic bacteria to heavy metals. *Curr. Microbiol.* 12, 273–276.
- Albracht, S.P.J., 1994. Nickel hydrogenases: in search of the active site. *Biochim. Biophys. Acta BBA - Bioenerg.* 1188, 167–204. doi:10.1016/0005-2728(94)90036-1
- Allwood, M.C., Kearney, M.C., 1998. Compatibility and stability of additives in parenteral nutrition admixtures. *Nutrition* 14, 697–706.
- Altaş, L., 2009. Inhibitory effect of heavy metals on methane-producing anaerobic granular sludge. *J. Hazard. Mater.* 162, 1551–1556. doi:10.1016/j.jhazmat.2008.06.048
- Amelunxen, R., Murdock, A.L., 1978. Microbial life at high temperatures: mechanisms and molecular aspects. *Microb. Life Extreme Environ.* 217–278.
- Andreesen, J.R., Ljungdahl, L.G., 1973. Formate dehydrogenase of *Clostridium thermoaceticum*: incorporation of selenium-75, and the effects of selenite, molybdate, and tungstate on the enzyme. *J. Bacteriol.* 116, 867–873.
- Angelidaki, I., Ahring, B., 1994. Anaerobic thermophilic digestion of manure at different ammonia loads: Effect of temperature. *Water Res.* 28, 727–731. doi:10.1016/0043-1354(94)90153-8
- Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., van Lier, J.B., 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Sci. Technol.* 59, 927. doi:10.2166/wst.2009.040
- Aquino, S.F., Stuckey, D.C., 2007. Bioavailability and toxicity of metal nutrients during anaerobic digestion. *J. Environ. Eng.* 133, 28–35.
- Artymiuk, P.J., Blake, C.C.F., Grace, D.E.P., Oatley, S.J., Phillips, D.C., Sternberg, M.J.E., 1979. Crystallographic studies of the dynamic properties of lysozyme. *Nature* 280, 563–568. doi:10.1038/280563a0
- Auerbach, G., Ostendorp, R., Prade, L., Korndörfer, I., Dams, T., Huber, R., Jaenicke, R., 1998. Lactate dehydrogenase from the hyperthermophilic bacterium *Thermotoga maritima*: the crystal structure at 2.1 Å resolution reveals strategies for intrinsic protein stabilization. *Structure* 6, 769–781.
- Babich, H., Stotzky, G., 1983. Toxicity of nickel to microbes: environmental aspects. *Adv. Appl. Microbiol.* 29, 195.
- Banks, C.J., Zhang, Y., Jiang, Y., Heaven, S., 2012. Trace element requirements for stable food waste digestion at elevated ammonia concentrations. *Bioresour. Technol.* 104, 127–135. doi:10.1016/j.biortech.2011.10.068
- Bauer, M.W., Kelly, R.M., 1998. The Family 1 β -Glucosidases from *Pyrococcus furiosus* and *Agrobacterium faecalis* Share a Common Catalytic Mechanism[†]. *Biochemistry (Mosc.)* 37, 17170–17178. doi:10.1021/bi9814944
- Bechdel, S.I., Honeywell, H.E., Dutcher, R.A., Knutsen, M.H., 1928. Synthesis of vitamin B in the rumen of the cow. *J. Biol. Chem.* 80, 231–238.
- Bennett, A., Rowe, R.I., Soch, N., Eckhert, C.D., 1999. Boron stimulates yeast (*Saccharomyces cerevisiae*) growth. *J. Nutr.* 129, 2236–2238.

611 Bertini, I., Luchinat, C., 1994. The reaction pathways of zinc enzymes and related biological catalysts.
 612 Bioinorg. Chem. 37–106.
 613 Bertram, P.A., Thauer, R.K., 1994. Thermodynamics of the formylmethanofuran dehydrogenase
 614 reaction in *Methanobacterium thermoautotrophicum*. Eur. J. Biochem. 226, 811–818.
 615 Beveridge, T.J., Doyle, R.J. (Eds.), 1989. Metal ions and bacteria. Wiley, New York.
 616 Boonyakitsombut, S., Kim, M.I., Ahn, Y.H., Speece, R.E., 2002. Degradation of propionate and its
 617 precursors: The role of nutrient supplementation. KSCE J. Civ. Eng. 6, 379–387.
 618 doi:10.1007/BF02841992
 619 Borja, R., Martín, A., Banks, C.J., Alonso, V., Chica, A., 1995. A kinetic study of anaerobic digestion of
 620 olive mill wastewater at mesophilic and thermophilic temperatures. Environ. Pollut. 88, 13–
 621 18. doi:10.1016/0269-7491(95)91043-K
 622 Bornstein, B.T., Barker, H.A., 1948. The Nutrition of *Clostridium kluyveri*. J. Bacteriol. 55, 223–230.
 623 Bourriaud, C., Robins, R.J., Martin, L., Kozlowski, F., Tenailleau, E., Cherbut, C., Michel, C., 2005.
 624 Lactate is mainly fermented to butyrate by human intestinal microfloras but inter-individual
 625 variation is evident. J. Appl. Microbiol. 99, 201–212. doi:10.1111/j.1365-2672.2005.02605.x
 626 Brahmachari, G., Demain, A.L., Adrio, J.L. (Eds.), 2017. Biotechnology of microbial enzymes:
 627 production, biocatalysis and industrial applications. Academic Press, an imprint of Elsevier,
 628 Amsterdam Boston; Heidelberg.
 629 Braun, R.D., 1991. Solubility of iron (II) carbonate at temperatures between 30 and 80. Talanta 38,
 630 205–211.
 631 Brock, T. D., Smith, D. W., Madigan, M. I., 1984. Biology of Organisms. Prentice Hall, Englewood Cliffs,
 632 N J, USA.
 633 Bryant, M., Tzeng, S., Robinson, I., Joyner Jr, A., 1971. Nutrient requirements of methanogenic
 634 bacteria. Urbana 101, 61801.
 635 Callander, I.J., Barford, J.P., 1983a. Precipitation, chelation, and the availability of metals as nutrients
 636 in anaerobic digestion. I. Methodology. Biotechnol. Bioeng. 25, 1947–1957.
 637 Callander, I.J., Barford, J.P., 1983b. Precipitation, chelation, and the availability of metals as nutrients
 638 in anaerobic digestion. II. Applications. Biotechnol. Bioeng. 25, 1959–1972.
 639 Carlsson, J., Griffith, C.J., 1974. Fermentation products and bacterial yields in glucose-limited and
 640 nitrogen-limited cultures of streptococci. Arch. Oral Biol. 19, 1105–1109. doi:10.1016/0003-
 641 9969(74)90238-6
 642 Cecchi, F., Pavan, P., Alvarez, J.M., Bassetti, A., Cozzolino, C., 1991. Anaerobic digestion of municipal
 643 solid waste: thermophilic vs. mesophilic performance at high solids. Waste Manag. Res. 9,
 644 305–315.
 645 Chen, M., Wolin, M.J., 1981. Influence of heme and vitamin B12 on growth and fermentations of
 646 *Bacteroides* species. J. Bacteriol. 145, 466–471.
 647 Chen, X., Schauder, S., Potier, N., Van Dorsselaer, A., Pelczar, I., Bassler, B.L., Hughson, F.M., 2002.
 648 Structural identification of a bacterial quorum-sensing signal containing boron. Nature 415,
 649 545–549.
 650 Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: A review.
 651 Bioresour. Technol. 99, 4044–4064. doi:10.1016/j.biortech.2007.01.057
 652 Cheong, D.Y., Hansen, C.L., Stevens, D.K., 2007. Production of bio-hydrogen by mesophilic anaerobic
 653 fermentation in an acid-phase sequencing batch reactor. Biotechnol Bioeng 96, 421–32.
 654 Chi, Y.-I., Martinez-Cruz, L.A., Jancarik, J., Swanson, R.V., Robertson, D.E., Kim, S.-H., 1999. Crystal
 655 structure of the β -glycosidase from the hyperthermophile *Thermosphaera aggregans*:
 656 insights into its activity and thermostability. FEBS Lett. 445, 375–383. doi:10.1016/S0014-
 657 5793(99)00090-3
 658 Clarens, M., Molleta, R., 1990. Kinetic studies of acetate fermentation by *Methanosarcina* sp. MSTA-
 659 1. Appl. Microbiol. Biotechnol. 33, 239–244.
 660 Coquelle, N., Fioravanti, E., Weik, M., Vellieux, F., Madern, D., 2007. Activity, Stability and Structural
 661 Studies of Lactate Dehydrogenases Adapted to Extreme Thermal Environments. J. Mol. Biol.
 662 374, 547–562. doi:10.1016/j.jmb.2007.09.049

- Cord-Ruwisch, R., Seitz, H.-J., Conrad, R., 1988. The capacity of hydrogenotrophic anaerobic bacteria to compete for traces of hydrogen depends on the redox potential of the terminal electron acceptor. *Arch. Microbiol.* 149, 350–357.
- D'Auria, S., Nucci, R., Rossi, M., Gryczynski, I., Gryczynski, Z., Lakowicz, J.R., 1999. The beta-glycosidase from the hyperthermophilic archaeon *Sulfolobus solfataricus*: enzyme activity and conformational dynamics at temperatures above 100 degrees C. *Biophys. Chem.* 81, 23–31.
- Dalman, L.H., 1937. The Solubility of Citric and Tartaric Acids in Water. *J. Am. Chem. Soc.* 59, 2547–2549. doi:10.1021/ja01291a018
- Daniel, R.M., Dines, M., Petach, H.H., 1996. The denaturation and degradation of stable enzymes at high temperatures. *Biochem. J.* 317 (Pt 1), 1–11.
- Daniels, L., Belay, N., BISWARUP, MUKHOPADAHYAY, 1984. Considerations for the use and large-scale growth of methanogenic bacteria. *Biotechnol. Bioeng.* 199–213.
- Danson, M.J., Hough, D.W., Russell, R.J., Taylor, G.L., Pearl, L., 1996. Enzyme thermostability and thermoactivity. *Protein Eng.* 9, 629–630.
- De Bok, F.A., Hagedoorn, P.-L., Silva, P.J., Hagen, W.R., Schiltz, E., Fritsche, K., Stams, A.J., 2003. Two W-containing formate dehydrogenases (CO₂-reductases) involved in syntrophic propionate oxidation by *Syntrophobacter fumaroxidans*. *Eur. J. Biochem.* 270, 2476–2485.
- De Kok, S., Meijer, J., van Loosdrecht, M.C., Kleerebezem, R., 2013. Impact of dissolved hydrogen partial pressure on mixed culture fermentations. *Appl Microbiol Biotechnol* 97, 2617–25.
- Demirel, B., Scherer, P., 2008. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review. *Rev. Environ. Sci. Biotechnol.* 7, 173–190.
- Dryden, L.P., Hartman, A.M., Bryant, M.P., Robinson, I.M., Moore, L.A., 1962a. Production of Vitamin B12 and Vitamin B12 Analogues by Pure Cultures of Ruminant Bacteria. *Nature* 195, 201–202. doi:10.1038/195201b0
- Dryden, L.P., Hartman, A.M., Bryant, M.P., Robinson, I.M., Moore, L.A., 1962b. Production of Vitamin B12 and Vitamin B12 Analogues by Pure Cultures of Ruminant Bacteria. *Nature* 195, 201–202. doi:10.1038/195201b0
- Duboc, P., Schill, N., Menoud, L., Van Gulik, W., Von Stockar, U., 1995. Measurements of sulfur, phosphorus and other ions in microbial biomass: influence on correct determination of elemental composition and degree of reduction. *J. Biotechnol.* 43, 145–158. doi:10.1016/0168-1656(95)00135-0
- Elias, M., Wieczorek, G., Rosenne, S., Tawfik, D.S., 2014. The universality of enzymatic rate–temperature dependency. *Trends Biochem. Sci.* 39, 1–7. doi:10.1016/j.tibs.2013.11.001
- Ensign, S.A., Hyman, M.R., Arp, D.J., 1993. In vitro activation of ammonia monooxygenase from *Nitrosomonas europaea* by copper. *J. Bacteriol.* 175, 1971–1980.
- Espinosa, A., 1995. Effect of trace metals on the anaerobic degradation of volatile fatty acids in molasses stillage. *Water Sci. Technol.* 32, 121–129. doi:10.1016/0273-1223(96)00146-1
- Fágáin, C.Ó., 1995. Understanding and increasing protein stability. *Biochim. Biophys. Acta BBA - Protein Struct. Mol. Enzymol.* 1252, 1–14. doi:10.1016/0167-4838(95)00133-F
- Fang, H.H., Liu, H., 2002. Effect of pH on hydrogen production from glucose by a mixed culture. *Bioresour. Technol.* 82, 87–93.
- Fang, H.H.P., Chan, O.C., 1997. Toxicity of Electroplating Metals on Benzoate-Degrading Granules. *Environ. Technol.* 18, 93–99. doi:10.1080/09593331808616516
- Fang, H.H.P., Hui, H.H., 1994. Effect of heavy metals on the methanogenic activity of starch-degrading granules. *Biotechnol. Lett.* 16, 1091–1096. doi:10.1007/BF01022409
- Fauque, G., Peck Jr, H., Moura, J., Huynh, B., Berlier, Y., DerVartanian, D., Teixeira, M., Przybyla, A., Lespinat, P., Moura, I., others, 1988. The three classes of hydrogenases from sulfate-reducing bacteria of the genus *Desulfovibrio*. *FEMS Microbiol. Rev.* 4, 299–344.
- Feller, G., 2010. Protein stability and enzyme activity at extreme biological temperatures. *J. Phys. Condens. Matter* 22, 323101. doi:10.1088/0953-8984/22/32/323101

- Feng, X.M., Karlsson, A., Svensson, B.H., Bertilsson, S., 2010. Impact of trace element addition on biogas production from food industrial waste - linking process to microbial communities: Biogas production from food industrial waste. *FEMS Microbiol. Ecol.* 74, 226–240. doi:10.1111/j.1574-6941.2010.00932.x
- Ferguson, S.J., 1994. Denitrification and its control. *Antonie Van Leeuwenhoek* 66, 89–110. doi:10.1007/BF00871634
- Fermoso, F.G., Collins, G., Bartacek, J., O’Flaherty, V., Lens, P., 2008. Acidification of methanol-fed anaerobic granular sludge bioreactors by cobalt deprivation: Induction and microbial community dynamics. *Biotechnol. Bioeng.* 99, 49–58. doi:10.1002/bit.21528
- Ferry, J.G., 2010. How to Make a Living by Exhaling Methane. *Annu. Rev. Microbiol.* 64, 453–473. doi:10.1146/annurev.micro.112408.134051
- Ferry, J.G., 1999. Enzymology of one-carbon metabolism in methanogenic pathways. *FEMS Microbiol. Rev.* 23, 13–38.
- Fisher, S., Buxbaum, L., Toth, K., Eisenstadt, E., Silver, S., 1973. Regulation of manganese accumulation and exchange in *Bacillus subtilis* W23. *J. Bacteriol.* 113, 1373–1380.
- Florencio, L., Field, J.A., Lettinga, G., 1994. Importance of cobalt for individual trophic groups in an anaerobic methanol-degrading consortium. *Appl. Environ. Microbiol.* 60, 227–234.
- Forsberg, C.W., 1978. Effects of heavy metals and other trace elements on the fermentative activity of the rumen microflora and growth of functionally important rumen bacteria. *Can. J. Microbiol.* 24, 298–306.
- Frauenfelder, H., Petsko, G.A., Tsernoglou, D., 1979. Temperature-dependent X-ray diffraction as a probe of protein structural dynamics. *Nature* 280, 558–563. doi:10.1038/280558a0
- Friedfeld, S.J., He, S., Tomson, M.B., 1998. The temperature and ionic strength dependence of the solubility product constant of ferrous phosphonate. *Langmuir* 14, 3698–3703.
- Gallert, C., Bauer, S., Winter, J., 1998. Effect of ammonia on the anaerobic degradation of protein by a mesophilic and thermophilic biowaste population. *Appl. Microbiol. Biotechnol.* 50, 495–501.
- GÃ-rio, F.M., Marcos, J.C., Amaral-CollaÃ-o, M.T., 1992. Transition metal requirement to express high level NAD⁺ dependent formate dehydrogenase from a serine-type methylotrophic bacterium. *FEMS Microbiol. Lett.* 97, 161–166. doi:10.1111/j.1574-6968.1992.tb05456.x
- Georlette, D., Damien, B., Blaise, V., Depiereux, E., Uversky, V.N., Gerday, C., Feller, G., 2003. Structural and functional adaptations to extreme temperatures in psychrophilic, mesophilic, and thermophilic DNA ligases. *J. Biol. Chem.* 278, 37015–37023. doi:10.1074/jbc.M305142200
- Ghasimi, D.S., Tao, Y., Kreuk, M., Zandvoort, M.H., Lier, J.B., 2015. Microbial population dynamics during long-term sludge adaptation of thermophilic and mesophilic sequencing batch digesters treating sewage fine sieved fraction at varying organic loading rates. *Biotechnol. Biofuels* 8, 1.
- Glass, J.B., Orphan, V.J., 2012. Trace Metal Requirements for Microbial Enzymes Involved in the Production and Consumption of Methane and Nitrous Oxide. *Front. Microbiol.* 3. doi:10.3389/fmicb.2012.00061
- Gonzalez-Gil, G., Jansen, S., Zandvoort, M.H., van Leeuwen, H.P., 2003. Effect of yeast extract on speciation and bioavailability of nickel and cobalt in anaerobic bioreactors. *Biotechnol. Bioeng.* 82, 134–142. doi:10.1002/bit.10551
- Gonzalez-Gil, G., Kleerebezem, R., Lettinga, G., 1999. Effects of nickel and cobalt on kinetics of methanol conversion by methanogenic sludge as assessed by on-line CH₄ monitoring. *Appl. Environ. Microbiol.* 65, 1789–1793.
- Greasham, R.L., Herber, W.K., 1997. Design and optimization of growth media. *Appl. Microb. Physiol.* IRL Press N. Y. 53–74.
- Grotenhuis, J., van Lier, J., Plugge, C., Stams, A., Zehnder, A., 1991. Effect of ethylene glycol-bis (β-aminoethyl ether)-N, N-tetraacetic acid (EGTA) on stability and activity of methanogenic granular sludge. *Appl. Microbiol. Biotechnol.* 36, 109–114.

767 Gujer, W., Zehnder, A., 1983. Conversion processes in anaerobic digestion. *Water Sci. Technol.* 15,
768 127–167.

769 Gupta, R., Gupta, N., Rath, P., 2004. Bacterial lipases: an overview of production, purification and
770 biochemical properties. *Appl. Microbiol. Biotechnol.* 64, 763–781. doi:10.1007/s00253-004-
771 1568-8

772 Hausinger, R.P., 1994. Nickel enzymes in microbes. *Sci. Total Environ.* 148, 157–166.
773 doi:10.1016/0048-9697(94)90392-1

774 Hausinger, R.P., 1987. Nickel utilization by microorganisms. *Microbiol. Rev.* 51, 22.

775 Hausinger, R.P., 1986. Purification of a nickel-containing urease from the rumen anaerobe
776 *Selenomonas ruminantium*. *J. Biol. Chem.* 261, 7866–7870.

777 Hawkes, F., Hussy, I., Kyazze, G., Dinsdale, R., Hawkes, D., 2007. Continuous dark fermentative
778 hydrogen production by mesophilic microflora: Principles and progress. *Int. J. Hydrog. Energy*
779 32, 172–184.

780 Heider, J., Bock, A., 1993. Selenium metabolism in micro-organisms. *Adv. Microb. Physiol.* 35, 71–
781 109.

782 Henderson, C., 1980. The influence of extracellular hydrogen on the metabolism of *Bacteroides*
783 *ruminicola*, *Anaerovibrio lipolytica* and *Selenomonas ruminantium*. *Microbiology* 119, 485–
784 491.

785 Hendlin, D., Ruger, M.L., 1950. The Effect of Cobalt on the Microbial Synthesis of LLD-active
786 Substances. *Science* 111, 541–542. doi:10.1126/science.111.2890.541

787 Henry, M.P., Donlon, B.A., Lens, P.N., Collieran, E.M., 1996. Use of anaerobic hybrid reactors for
788 treatment of synthetic pharmaceutical wastewaters containing organic solvents. *J. Chem.*
789 *Technol. Biotechnol.* 66, 251–264.

790 Henze, M., 2008. Biological wastewater treatment: principles, modelling and design. IWA publishing.

791 Henze, M., Harremoës, P., 1983. Anaerobic treatment of wastewater in fixed film reactors—a
792 literature review. *Water Sci. Technol.* 15, 1–101.

793 Hill, M., 1997. Intestinal flora and endogenous vitamin synthesis. *Eur. J. Cancer Prev.* 6, S43–S45.

794 Hobson, P.N., Stewart, C.S., 1997. The Rumen Microbial Ecosystem. Springer Netherlands, Dordrecht.

795 Holmes, M.A., Matthews, B.W., 1981. Binding of hydroxamic acid inhibitors to crystalline thermolysin
796 suggests a pentacoordinate zinc intermediate in catalysis. *Biochemistry (Mosc.)* 20, 6912–
797 6920.

798 Hopfner, K.-P., Eichinger, A., Engh, R.A., Laue, F., Ankenbauer, W., Huber, R., Angerer, B., 1999.
799 Crystal structure of a thermostable type B DNA polymerase from *Thermococcus gorgonarius*.
800 *Proc. Natl. Acad. Sci.* 96, 3600–3605.

801 Hubbert, F., Cheng, E., Burroughs, W., 1958. Mineral Requirement of Rumen Microorganisms for
802 Cellulose Digestion. *J. Anim. Sci.* 17, 559–568.

803 Huber, R., 1979. Conformational flexibility in protein molecules. *Nature* 280, 538–538.

804 Huber, R., Bennett, W.S., 1983. Functional significance of flexibility in proteins. *Biopolymers* 22, 261–
805 279.

806 Hughes, M.N., Poole, R.K., 1991. Metal speciation and microbial growth—the hard(and soft) facts.
807 *Microbiology* 137, 725–734.

808 Inatomi, K.-I., 1986. Characterization and purification of the membrane-bound ATPase of the
809 archaeobacterium *Methanosarcina barkeri*. *J. Bacteriol.* 167, 837–841.

810 Isupov, M.N., Fleming, T.M., Dalby, A.R., Crowhurst, G.S., Bourne, P.C., Littlechild, J.A., 1999. Crystal
811 structure of the glyceraldehyde-3-phosphate dehydrogenase from the hyperthermophilic
812 archaeon *Sulfolobus solfataricus*. *J. Mol. Biol.* 291, 651–660.

813 Jaenicke, R., 1996. Stability and folding of ultrastable proteins: eye lens crystallins and enzymes from
814 thermophiles. *FASEB J.* 10, 84–92.

815 Jaenicke, R., 1991. Protein stability and molecular adaptation to extreme conditions. *Eur. J. Biochem.*
816 202, 715–728. doi:10.1111/j.1432-1033.1991.tb16426.x

817 Jarrell, K.F., Kalmokoff, M.L., 1988. Nutritional requirements of the methanogenic archaeobacteria.
818 *Can. J. Microbiol.* 34, 557–576.

Jiang, B., 2006. The effect of trace elements on the metabolism of methanogenic consortia. s.n.], S.I.

Jones, J.B., Stadtman, T.C., 1977. Methanococcus vannielii: culture and effects of selenium and tungsten on growth. *J. Bacteriol.* 130, 1404.

Kabay, N., Bryjak, M., Hilal, N., 2015. Boron separation processes. Elsevier.

Karadag, D., Puhakka, J.A., 2010. Enhancement of anaerobic hydrogen production by iron and nickel. *Int. J. Hydrog. Energy* 35, 8554–8560. doi:10.1016/j.ijhydene.2010.04.174

Kayhanian, M., Rich, D., 1995. Pilot-scale high solids thermophilic anaerobic digestion of municipal solid waste with an emphasis on nutrient requirements. *Biomass Bioenergy* 8, 433–444. doi:10.1016/0961-9534(95)00043-7

Kenealy, W.R., Zeikus, J., 1982. Characterization and function of phosphoenolpyruvate carboxylase in *Methanobacterium thermoautotrophicum*. *FEMS Microbiol Lett* 14, 7–10.

Kim, M., Ahn, Y.-H., Speece, R., 2002. Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic. *Water Res.* 36, 4369–4385. doi:10.1016/S0043-1354(02)00147-1

Kirby, T.W., Lancaster, J.R., Fridovich, I., 1981. Isolation and characterization of the iron-containing superoxide dismutase of *Methanobacterium bryantii*. *Arch. Biochem. Biophys.* 210, 140–148. doi:10.1016/0003-9861(81)90174-0

Koster, I.W., Lettinga, G., 1984. The influence of ammonium-nitrogen on the specific activity of pelletized methanogenic sludge. *Agric. Wastes* 9, 205–216. doi:10.1016/0141-4607(84)90080-5

Krishna Ravuri, H., 2013. Role of factors influencing on anaerobic process for production of bio hydrogen: Future fuel. *Int. J. Adv. Chem.* 1, 31–38.

Latt, S.A., Holmquist, B., Vallee, B.L., 1969. Thermolysin: a zinc metalloenzyme. *Biochem. Biophys. Res. Commun.* 37, 333–339.

LeBlanc, J.G., Milani, C., de Giori, G.S., Sesma, F., van Sinderen, D., Ventura, M., 2013. Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Curr. Opin. Biotechnol.* 24, 160–168. doi:10.1016/j.copbio.2012.08.005

Li, C., Fang, H.H.P., 2007. Inhibition of heavy metals on fermentative hydrogen production by granular sludge. *Chemosphere* 67, 668–673. doi:10.1016/j.chemosphere.2006.11.005

Lier, J. van, 1995. Thermophilic anaerobic wastewater treatment, temperature aspects and process stability. Ph. D. Thesis, Department of Environmental Technology, Wageningen University, the Netherlands.

Lin, C.-Y., 1993. Effect of heavy metals on acidogenesis in anaerobic digestion. *Water Res.* 27, 147–152. doi:10.1016/0043-1354(93)90205-V

Lin, C.-Y., 1992. Effect of heavy metals on volatile fatty acid degradation in anaerobic digestion. *Water Res.* 26, 177–183. doi:10.1016/0043-1354(92)90217-R

Lin, C.-Y., Chen, C.-C., 1999. Effect of heavy metals on the methanogenic UASB granule. *Water Res.* 33, 409–416.

Lin, C.-Y., Chen, C.-C., 1997. Toxicity-resistance of sludge biogranules to heavy metals. *Biotechnol. Lett.* 19, 557–560.

Linehan, B., Scheifinger, C., Wolin, M., 1978. Nutritional requirements of *Selenomonas ruminantium* for growth on lactate, glycerol, or glucose. *Appl. Environ. Microbiol.* 35, 317–322.

Lipscomb, J.D., 1994. Biochemistry of the soluble methane monooxygenase. *Annu. Rev. Microbiol.* 48, 371–399.

Lipscomb, W.N., 1970. Structure and mechanism in the enzymic activity of carboxypeptidase A and relations to chemical sequence. *Acc. Chem. Res.* 3, 81–89.

Ljungdahl, L.G., 1979. Physiology of thermophilic bacteria. *Adv. Microb. Physiol.* 19, 149–243.

Ljungdahl, L.G., Andreesen, J.R., 1975. Tungsten, a component of active formate dehydrogenase from *Clostridium thermoaceticum*. *FEBS Lett.* 54, 279–282. doi:10.1016/0014-5793(75)80092-5

Lytle, B.L., Volkman, B.F., Westler, W.M., Wu, J.H.D., 2000. Secondary Structure and Calcium-Induced Folding of the *Clostridium thermocellum* Dockerin Domain Determined by NMR Spectroscopy. *Arch. Biochem. Biophys.* 379, 237–244. doi:10.1006/abbi.2000.1882

- Madigan, M.T., Martinko, J.M., Parker, J., others, 1997. Brock biology of microorganisms. prentice hall Upper Saddle River, NJ.
- Maes, D., Zeelen, J.P., Thanki, N., Beaucamp, N., Alvarez, M., Thi, M.H.D., Backmann, J., Martial, J.A., Wyns, L., Jaenicke, R., others, 1999. The crystal structure of triosephosphate isomerase (TIM) from *Thermotoga maritima*: a comparative thermostability structural analysis of ten different TIM structures. *Proteins Struct. Funct. Bioinforma.* 37, 441–453.
- Mahadevan, S., Sauer, F.D., Erfle, J.D., 1977. Purification and properties of urease from bovine rumen. *Biochem J* 163, 495–501.
- Maret, W., Wedd, A., 2014. Binding, transport and storage of metal ions in biological cells.
- Martinez, A., Church, D.C., 1970. Effect of various mineral elements on in vitro rumen cellulose digestion. *J. Anim. Sci.* 31, 982–990.
- McCarty, P.L., Vath, C.A., 1962. Volatile acid digestion at high loading rates. *Int J Air Water Pollut* 6, 65–73.
- Metcalf, E., 2005. Wastewater Engineering Treatment and Reuse, Metcalf & Eddy. Inc.
- Milanovich, F.P., Wilson, D.W., Yeh, Y., 1975. Detoxifying effect of yellow substance on *E. coli* in media containing copper. *Nature* 253, 460–461. doi:10.1038/253460a0
- Miura, H., Horiguchi, M., Matsumoto, T., 1980. Nutritional interdependence among rumen bacteria, *Bacteroides amylophilus*, *Megasphaera elsdenii*, and *Ruminococcus albus*. *Appl. Environ. Microbiol.* 40, 294–300.
- Moestedt, J., Nordell, E., Shakeri Yekta, S., Lundgren, J., Martí, M., Sundberg, C., Ejlertsson, J., Svensson, B.H., Björn, A., 2015. Effects of trace element addition on process stability during anaerobic co-digestion of OFMSW and slaughterhouse waste. *Waste Manag.* doi:10.1016/j.wasman.2015.03.007
- Mulder, R., 1990. Aggregation in an acidifying reactor. [Druck] Quint.
- Mulrooney, S.B., Hausinger, R.P., 2003. Nickel uptake and utilization by microorganisms. *FEMS Microbiol. Rev.* 27, 239–261. doi:10.1016/S0168-6445(03)00042-1
- Muñoz Sierra, J.D., Lafita, C., Gabaldón, C., Spanjers, H., van Lier, J.B., 2017. Trace metals supplementation in anaerobic membrane bioreactors treating highly saline phenolic wastewater. *Bioresour. Technol.* 234, 106–114. doi:10.1016/j.biortech.2017.03.032
- N. Jisha, V., B. Smitha, R., Pradeep, S., Sreedevi, S., N. Unni, K., Sajith, S., Priji, P., Sarath Josh, M., Benjamin, S., 2013. Versatility of microbial proteases. *Adv. Enzyme Res.* 01, 39–51. doi:10.4236/aer.2013.13005
- Nandan, R., Tondwalkar, V., Ray, P.K., 1990. Biomethanation of spent wash: Heavy metal inhibition of methanogenesis in synthetic medium. *J. Ferment. Bioeng.* 69, 276–281.
- Nelson, M.J., Ferry, J.G., 1984. Carbon monoxide-dependent methyl coenzyme M methylreductase in acetotrophic *Methosarcina* spp. *J. Bacteriol.* 160, 526–532.
- Neumann, A., Wohlfarth, G., Diekert, G., 1996. Purification and characterization of tetrachloroethene reductive dehalogenase from *Dehalosporillum multivorans*. *J. Biol. Chem.* 271, 16515–16519.
- Nishio, N., Kakizono, T., Silveira, R.G., Takemoto, S., Nagai, S., 1992. Nutrient control by the gas evolution in methanogenesis of methanol by *Methanosarcina barkeri*. *J. Ferment. Bioeng.* 73, 481–485. doi:10.1016/0922-338X(92)90142-H
- Nishio, N., Nagai, S., others, 1991. Effects of trace metal ions on the growth, homoacetogenesis and corrinoid production by *Clostridium thermoaceticum*. *J. Ferment. Bioeng.* 71, 181–185.
- Nörtemann, B., 2005. Biodegradation of Chelating Agents: EDTA, DTPA, PDTA, NTA, and EDDS, in: Nowack, B., VanBriesen, J.M. (Eds.), *Biogeochemistry of Chelating Agents*. American Chemical Society, Washington, DC, pp. 150–170.
- Oh, H., Wee, Y.-J., Yun, J.-S., Ryu, H.-W., 2003. Lactic acid production through cell-recycle repeated-batch bioreactor, in: *Biotechnology for Fuels and Chemicals*. Springer, pp. 603–613.
- Oh, Y.-K., Kim, S.H., Kim, M.-S., Park, S., 2004. Thermophilic biohydrogen production from glucose with trickling biofilter. *Biotechnol. Bioeng.* 88, 690–698. doi:10.1002/bit.20269

- Ortner, M., Rameder, M., Rachbauer, L., Bochmann, G., Fuchs, W., 2015. Bioavailability of essential trace elements and their impact on anaerobic digestion of slaughterhouse waste. *Biochem. Eng. J.* 99, 107–113. doi:10.1016/j.bej.2015.03.021
- Osuna, M.B., Iza, J., Zandvoort, M., Lens, P.N.L., 2003. Essential metal depletion in an anaerobic reactor. *Water Sci. Technol. J. Int. Assoc. Water Pollut. Res.* 48, 1–8.
- Öztürk, M., 1991. Conversion of acetate, propionate and butyrate to methane under thermophilic conditions in batch reactors. *Water Res.* 25, 1509–1513. doi:10.1016/0043-1354(91)90181-O
- Pappenheimer, A.M., Shaskan, E., 1944. Effect of iron on carbohydrate metabolism of *Clostridium welchii*. *J. Biol. Chem.* 155, 265–275.
- Patel, V.B., Patel, A.R., Patel, M.C., Madamwar, D.B., 1993. Effect of metals on anaerobic digestion of water hyacinth-cattle dung. *Appl. Biochem. Biotechnol.* 43, 45–50.
- Pavlostathis, S.G., Giraldo-Gomez, E., 1991. Kinetics of anaerobic treatment: A critical review. *Crit. Rev. Environ. Control* 21, 411–490. doi:10.1080/10643389109388424
- Paynter, M., Elsdon, S., 1970. Mechanism of propionate formation by *Selenomonas ruminantium*, a rumen micro-organism. *J. Gen. Microbiol.* 61, 1–7.
- Pedersen, D., Sayler, G., 1981. Methanogenesis in freshwater sediments: inherent variability and effects of environmental contaminants. *Can. J. Microbiol.* 27, 198–205.
- Perry, R.D., Silver, S., 1982. Cadmium and manganese transport in *Staphylococcus aureus* membrane vesicles. *J. Bacteriol.* 150, 973–976.
- Plugge, C.M., Jiang, B., de Bok, F.A.M., Tsai, C., Stams, A.J.M., 2009. Effect of tungsten and molybdenum on growth of a syntrophic coculture of *Syntrophobacter fumaroxidans* and *Methanospirillum hungatei*. *Arch. Microbiol.* 191, 55–61. doi:10.1007/s00203-008-0428-9
- Pobeheim, H., Munk, B., Johansson, J., Guebitz, G.M., 2010. Influence of trace elements on methane formation from a synthetic model substrate for maize silage. *Bioresour. Technol.* 101, 836–839. doi:10.1016/j.biortech.2009.08.076
- Pritchard, G.G., Coolbear, T., 1993. The physiology and biochemistry of the proteolytic system in lactic acid bacteria. *FEMS Microbiol. Rev.* 12, 179–206.
- Qiang, H., Lang, D.-L., Li, Y.-Y., 2012. High-solid mesophilic methane fermentation of food waste with an emphasis on Iron, Cobalt, and Nickel requirements. *Bioresour. Technol.* 103, 21–27. doi:10.1016/j.biortech.2011.09.036
- Qiang, H., Niu, Q., Chi, Y., Li, Y., 2013. Trace metals requirements for continuous thermophilic methane fermentation of high-solid food waste. *Chem. Eng. J.* 222, 330–336. doi:10.1016/j.cej.2013.02.076
- Rao, M.B., Tanksale, A.M., Ghatge, M.S., Deshpande, V.V., 1998. Molecular and biotechnological aspects of microbial proteases. *Microbiol. Mol. Biol. Rev.* 62, 597–635.
- Ridge, P.G., Zhang, Y., Gladyshev, V.N., 2008. Comparative Genomic Analyses of Copper Transporters and Cuproproteomes Reveal Evolutionary Dynamics of Copper Utilization and Its Link to Oxygen. *PLoS ONE* 3, e1378. doi:10.1371/journal.pone.0001378
- Rittmann, B.E., McCarty, P.L., 2001. *Environmental biotechnology: principles and applications*, McGraw-Hill series in water resources and environmental engineering. McGraw-Hill, Boston.
- Rivers, S., Umney, N., 2003. *Conservation of furniture*. Butterworth Heinemann, Oxford, England; Burlington, MA.
- Röling, W.F., Van Verseveld, H.W., 1997. Growth, maintenance and fermentation pattern of the salt-tolerant lactic acid bacterium *Tetragenococcus halophila* in anaerobic glucose limited retention cultures. *Antonie Van Leeuwenhoek* 72, 239–243.
- Russell, J., 1992. Another explanation for the toxicity of fermentation acids at low pH: anion accumulation versus uncoupling. *J. Appl. Microbiol.* 73, 363–370.
- Russell, J.B., Diez-Gonzalez, F., 1997. The effects of fermentation acids on bacterial growth. *Adv. Microb. Physiol.* 39, 205–234.
- Russell, R.J.M., Ferguson, J.M.C., Hough, D.W., Danson, M.J., Taylor, G.L., 1997. The Crystal Structure of Citrate Synthase from the Hyperthermophilic Archaeon *Pyrococcus furiosus* at 1.9 Å Resolution^{†, ‡}. *Biochemistry (Mosc.)* 36, 9983–9994. doi:10.1021/bi9705321

973 Sanchez, J.M., Valle, L., Rodriguez, F., Morinigo, M.A., Borrego, J.J., 1996. Inhibition of
 974 methanogenesis by several heavy metals using pure cultures. Lett. Appl. Microbiol. 23, 439–
 975 444.
 976 Sauer, K., Thauer, R.K., 2000. Methyl-coenzyme M formation in methanogenic archaea: Involvement
 977 of zinc in coenzyme M activation. Eur. J. Biochem. 267, 2498–2504. doi:10.1046/j.1432-
 978 1327.2000.01245.x
 979 Sawers, G., 1994. The hydrogenases and formate dehydrogenases of *Escherichia coli*. Antonie Van
 980 Leeuwenhoek 66, 57–88. doi:10.1007/BF00871633
 981 Schauer, N.L., Ferry, J.G., 1982. Properties of formate dehydrogenase in *Methanobacterium*
 982 *formicum*. J. Bacteriol. 150, 1–7.
 983 Scheifinger, C., Linehan, B., Wolin, M., 1975. H₂ production by *Selenomonas ruminantium* in the
 984 absence and presence of methanogenic bacteria. Appl. Microbiol. 29, 480–483.
 985 Scherer, P., Lippert, H., Wolff, G., 1983. Composition of the major elements and trace elements of 10
 986 methanogenic bacteria determined by inductively coupled plasma emission spectrometry.
 987 Biol. Trace Elem. Res. 5, 149–163.
 988 Schindelin, H., Kisker, C., Schlessman, J.L., Howard, J.B., Rees, D.C., 1997. Structure of ADP·AlF₄ –
 989 stabilized nitrogenase complex and its implications for signal transduction. Nature 387, 370–
 990 376. doi:10.1038/387370a0
 991 Schönheit, P., Brandis, A., Thauer, R.K., 1979a. Ferredoxin degradation in growing *Clostridium*
 992 *pasteurianum* during periods of iron deprivation. Arch. Microbiol. 120, 73–76.
 993 Schönheit, P., Moll, J., Thauer, R.K., 1979b. Nickel, cobalt, and molybdenum requirement for growth
 994 of *Methanobacterium thermoautotrophicum*. Arch. Microbiol. 123, 105–107.
 995 doi:10.1007/BF00403508
 996 Schwarz, W.H., 2001. The cellulosome and cellulose degradation by anaerobic bacteria. Appl.
 997 Microbiol. Biotechnol. 56, 634–649. doi:10.1007/s002530100710
 998 Scott, H.W., Dehority, B.A., 1965. Vitamin requirements of several cellulolytic rumen bacteria. J.
 999 Bacteriol. 89, 1169–1175.
 1000 Seravalli, J., Gu, W., Tam, A., Strauss, E., Begley, T.P., Cramer, S.P., Ragsdale, S.W., 2003. Functional
 1001 copper at the acetyl-CoA synthase active site. Proc. Natl. Acad. Sci. 100, 3689–3694.
 1002 Sillén, L.G., Martell, A.E., Bjerrum, J., 1964. Stability constants of metal-ion complexes. Chemical
 1003 Society London.
 1004 Silley, P., Armstrong, D.G., 1984. Changes in metabolism of the rumen bacterium *Streptococcus bovis*
 1005 H13/1 resulting from alteration in dilution rate and glucose supply per unit time. J. Appl.
 1006 Bacteriol. 57, 345–353. doi:10.1111/j.1365-2672.1984.tb01400.x
 1007 Solis, G.J., Alonso, E., Riesco, P., 2002. Distribution of metal extractable fractions during anaerobic
 1008 sludge treatment in southern Spain WWTPs. Water. Air. Soil Pollut. 140, 139–156.
 1009 Speece, R.E., 1996. Anaerobic biotechnology for industrial wastewaters. Archae Press, Nashville,
 1010 Tenn.
 1011 Speece, R.E., 1988. A survey of municipal anaerobic sludge digesters and diagnostic activity assays.
 1012 Water Res. 22, 365–372. doi:10.1016/S0043-1354(88)90260-6
 1013 Speece, R.E., McCarty, P.L., 1964. Nutrient requirements and biological solids accumulation in
 1014 anaerobic digestion. Adv. Water Pollut. Res. 2, 305–322.
 1015 Speece, R.E., Parkin, G.F., Gallagher, D., 1983. Nickel stimulation of anaerobic digestion. Water Res.
 1016 17, 677–683. doi:10.1016/0043-1354(83)90237-3
 1017 Stadtman, T.C., 1980. Biological functions of selenium. Trends Biochem. Sci. 5, 203–206.
 1018 doi:10.1016/S0968-0004(80)80008-9
 1019 Strobel, H.J., 1992. Vitamin B₁₂-dependent propionate production by the ruminal bacterium
 1020 *Prevotella ruminicola* 23. Appl. Environ. Microbiol. 58, 2331–2333.
 1021 Tahirov, T.H., Oki, H., Tsukihara, T., Ogasahara, K., Yutani, K., Ogata, K., Izu, Y., Tsunasawa, S., Kato, I.,
 1022 1998. Crystal structure of methionine aminopeptidase from hyperthermophile, *Pyrococcus*
 1023 *furiosus*. J. Mol. Biol. 284, 101–124.

1024 Takashima, M., Shimada, K., Speece, R.E., 2011. Minimum Requirements for Trace Metals (Iron,
 1025 Nickel, Cobalt, and Zinc) in Thermophilic and Mesophilic Methane Fermentation from
 1026 Glucose. *Water Environ. Res.* 83, 339–346. doi:10.2175/106143010X12780288628895
 1027 Takashima, M., Speece, R., 1989. Mineral nutrient requirements for high-rate methane fermentation
 1028 of acetate at low SRT. *Res. J. Water Pollut. Control Fed.* 1645–1650.
 1029 Takashima, M., Speece, R.E., Parkin, G.F., 1990. Mineral requirements for methane fermentation.
 1030 *Crit. Rev. Environ. Control* 19, 465–479. doi:10.1080/10643389009388378
 1031 Tchobanoglous, G., Burton, F.L., Stensel, H.D., Metcalf & Eddy (Eds.), 2003. Wastewater engineering:
 1032 treatment and reuse, 4th ed. ed, McGraw-Hill series in civil and environmental engineering.
 1033 McGraw-Hill, Boston.
 1034 Temudo, M.F., Kleerebezem, R., van Loosdrecht, M., 2007. Influence of the pH on (open) mixed
 1035 culture fermentation of glucose: a chemostat study. *Biotechnol Bioeng* 98, 69–79.
 1036 Thauer, R.K., Diekert, G., Schönheit, P., 1980. Biological role of nickel. *Trends Biochem. Sci.* 5, 304–
 1037 306. doi:10.1016/0968-0004(80)90166-8
 1038 Thompson, R.C., 1942. Synthesis of B vitamins by bacteria in pure culture. *Univ Tex. Pub* 87–96.
 1039 Tijhuis, L., Van Loosdrecht, M.C.M., Heijnen, J.J., 1993. A thermodynamically based correlation for
 1040 maintenance gibbs energy requirements in aerobic and anaerobic chemotrophic growth.
 1041 *Biotechnol. Bioeng.* 42, 509–519.
 1042 Tomlinson, N., 1954a. Carbon dioxide and acetate utilization by *Clostridium kluyveri* II. Synthesis of
 1043 amino acids. *J. Biol. Chem.* 209, 597–603.
 1044 Tomlinson, N., 1954b. Carbon Dioxide and Acetate Utilization by *Clostridium kluyveri* III. A new Path
 1045 of Glutamic Acid Synthesis. *J. Biol. Chem.* 209, 605–609.
 1046 Tomlinson, N., Barker, H.A., 1954. Carbon Dioxide and Acetate Utilization by *Clostridium kluyveri* I.
 1047 Influence of Nutritional Conditions on Utilization Patterns. *J. Biol. Chem.* 209, 585–595.
 1048 Turton, L.J., Drucker, D.B., Hillier, V.F., Ganguli, L.A., 1983. Effect of eight growth media upon
 1049 fermentation profiles of ten anaerobic bacteria. *J. Appl. Bacteriol.* 54, 295–304.
 1050 Uemura, S., 2010. Mineral requirements for mesophilic and thermophilic anaerobic digestion of
 1051 organic solid waste.
 1052 Vallee, B.L., Ulmer, D.D., 1972. Biochemical Effects of Mercury, Cadmium, and Lead. *Annu. Rev.*
 1053 *Biochem.* 41, 91–128. doi:10.1146/annurev.bi.41.070172.000515
 1054 Van der Meijden, P., Te Brömmelstroet, B.W., Poirot, C.M., van der Drift, C., Vogels, G.D., 1984.
 1055 Purification and properties of methanol: 5-hydroxybenzimidazolylcobamide
 1056 methyltransferase from *Methanosarcina barkeri*. *J. Bacteriol.* 160, 629–635.
 1057 Varley, P.G., Pain, R.H., 1991. Relation between stability, dynamics and enzyme activity in 3-
 1058 phosphoglycerate kinases from yeast and *Thermus thermophilus*. *J. Mol. Biol.* 220, 531–538.
 1059 doi:10.1016/0022-2836(91)90028-5
 1060 Veeken, A., 1999. Removal of heavy metals from sewage sludge by extraction with organic acids.
 1061 *Water Sci. Technol.* 40, 129–136. doi:10.1016/S0273-1223(99)00373-X
 1062 Vieille, C., Hess, J.M., Kelly, R.M., Zeikus, J.G., 1995. xylA cloning and sequencing and biochemical
 1063 characterization of xylose isomerase from *Thermotoga neapolitana*. *Appl. Environ. Microbiol.*
 1064 61, 1867–1875.
 1065 Vintiloiu, A., Boxriker, M., Lemmer, A., Oechsner, H., Jungbluth, T., Mathies, E., Ramhold, D., 2013.
 1066 Effect of ethylenediaminetetraacetic acid (EDTA) on the bioavailability of trace elements
 1067 during anaerobic digestion. *Chem. Eng. J.* 223, 436–441. doi:10.1016/j.cej.2013.02.104
 1068 Wang, D.I., Cooney, C.L., Demain, A.L., Dunnill, P., Humphrey, A.E., Lilly, M.D., 1979. Fermentation
 1069 and enzyme technology. Wiley New York.
 1070 Weimer, P., Zeikus, J., 1978. Acetate metabolism in *Methanosarcina barkeri*. *Arch. Microbiol.* 119,
 1071 175–182.
 1072 White, H., Simon, H., 1992. The role of tungstate and/or molybdate in the formation of aldehyde
 1073 oxidoreductase in *Clostridium thermoaceticum* and other acetogens; immunological
 1074 distances of such enzymes. *Arch. Microbiol.* 158, 81–84.

1075 White, M., Shalita, Z., Marcus, D., Reuveny, S., 1990. A systematic route for optimization of media
 1076 composition and growth conditions. *Biol. Recomb. Microorg. Anim. Cells Prod. Recovery*
 1077 White MD Reuveny Shafferman Eds 101–116.
 1078 Whitman, W.B., Ankwarda, E., Wolfe, R.S., 1982. Nutrition and carbon metabolism of
 1079 *Methanococcus voltae*. *J. Bacteriol.* 149, 852–863.
 1080 Wiegant, W., 1986. Thermophilic anaerobic digestion for waste and wastewater treatment.
 1081 Landbouwniversiteit te Wageningen.
 1082 Wolfe, R., 1971. Microbial formation of methane. *Adv. Microb. Physiol.* 6, 107–146.
 1083 Wolf-Watz, M., Thai, V., Henzler-Wildman, K., Hadjipavlou, G., Eisenmesser, E.Z., Kern, D., 2004.
 1084 Linkage between dynamics and catalysis in a thermophilic-mesophilic enzyme pair. *Nat.*
 1085 *Struct. Mol. Biol.* 11, 945–949. doi:10.1038/nsmb821
 1086 Wolin, E.A., Wolin, M.J., Wolfe, R.S., 1963. Formation of Methane by Bacterial Extracts. *J. Biol. Chem.*
 1087 238, 2882–2886.
 1088 Worm, P., Feroso, F.G., Lens, P.N.L., Plugge, C.M., 2009. Decreased activity of a propionate
 1089 degrading community in a UASB reactor fed with synthetic medium without molybdenum,
 1090 tungsten and selenium. *Enzyme Microb. Technol.* 45, 139–145.
 1091 doi:10.1016/j.enzmictec.2009.02.001
 1092 Wrba, A., Schweiger, A., Schultes, V., Jaenicke, R., Zavodszky, P., 1990. Extremely thermostable D-
 1093 glyceraldehyde-3-phosphate dehydrogenase from the eubacterium *Thermotoga maritima*.
 1094 *Biochemistry (Mosc.)* 29, 7584–7592.
 1095 Ye, K., Shijo, M., Jin, S., Shimizu, K., 1996. Efficient production of vitamin B12 from propionic acid
 1096 bacteria under periodic variation of dissolved oxygen concentration. *J. Ferment. Bioeng.* 82,
 1097 484–491. doi:10.1016/S0922-338X(97)86988-7
 1098 Zandvoort, M.H., Geerts, R., Lettinga, G., Lens, P.N.L., 2002. Effect of Long-Term Cobalt Deprivation
 1099 on Methanol Degradation in a Methanogenic Granular Sludge Bioreactor. *Biotechnol. Prog.*
 1100 18, 1233–1239. doi:10.1021/bp020078e
 1101 Zandvoort, M.H., Gieteling, J., Lettinga, G., Lens, P.N.L., 2004. Stimulation of methanol degradation in
 1102 UASB reactors: In situ versus pre-loading cobalt on anaerobic granular sludge. *Biotechnol.*
 1103 *Bioeng.* 87, 897–904. doi:10.1002/bit.20200
 1104 Zandvoort, M.H., Osuna, M.B., Geerts, R., Lettinga, G., Lens, P.N.L., 2002. Effect of nickel deprivation
 1105 on methanol degradation in a methanogenic granular sludge bioreactor. *J. Ind. Microbiol.*
 1106 *Biotechnol.* 29, 268–274. doi:10.1038/sj.jim.7000311
 1107 Zandvoort, M.H., van Hullebusch, E.D., Feroso, F.G., Lens, P.N.L., 2006. Trace Metals in Anaerobic
 1108 Granular Sludge Reactors: Bioavailability and Dosing Strategies. *Eng. Life Sci.* 6, 293–301.
 1109 doi:10.1002/elsc.200620129
 1110 Zavodszky, P., Kardos, J., Svingor, Á., Petsko, G.A., 1998. Adjustment of conformational flexibility is a
 1111 key event in the thermal adaptation of proteins. *Proc. Natl. Acad. Sci.* 95, 7406–7411.
 1112 Zayed, G., Winter, J., 2000. Inhibition of methane production from whey by heavy metals--protective
 1113 effect of sulfide. *Appl. Microbiol. Biotechnol.* 53, 726–731.
 1114 Zehnder, A.J., Wuhrmann, K., 1977. Physiology of a *Methanobacterium* strain AZ. *Arch. Microbiol.*
 1115 111, 199–205.
 1116 Zellner, G., Alten, C., Stackebrandt, E., De Macario, E.C., Winter, J., 1987. Isolation and
 1117 characterization of *Methanocorpusculum parvum*, gen. nov., spec. nov., a new tungsten
 1118 requiring, coccoid methanogen. *Arch. Microbiol.* 147, 13–20.
 1119 Zellner, G., Winter, J., 1987. Growth promoting effect of tungsten on methanogens and incorporation
 1120 of tungsten-185 into cells. *FEMS Microbiol. Lett.* 40, 81–87.
 1121 Zhang, J., Greasham, R., 1999. Chemically defined media for commercial fermentations. *Appl.*
 1122 *Microbiol. Biotechnol.* 51, 407–421.
 1123 Zhang, Y., Zhang, Z., Suzuki, K., Maekawa, T., 2003. Uptake and mass balance of trace metals for
 1124 methane producing bacteria. *Biomass Bioenergy* 25, 427–433. doi:10.1016/S0961-
 1125 9534(03)00012-6

1126 Zheng, X.-J., Yu, H.-Q., 2004. Biological Hydrogen Production by Enriched Anaerobic Cultures in the
 1127 Presence of Copper and Zinc. *J. Environ. Sci. Health Part A* 39, 89–101. doi:10.1081/ESE-
 1128 120027370

1129 Zinder, S.H., 1990. Conversion of acetic acid to methane by thermophiles. *FEMS Microbiol. Lett.* 75,
 1130 125–137. doi:10.1111/j.1574-6968.1990.tb04090.x

1131 Zoetemeyer, R., Matthijsen, A., Cohen, A., Boelhouwer, C., 1982. Product inhibition in the acid
 1132 forming stage of the anaerobic digestion process. *Water Res.* 16, 633–639.

1133 Zwickl, P., Fabry, S., Bogedain, C., Haas, A., Hensel, R., 1990. Glyceraldehyde-3-phosphate
 1134 dehydrogenase from the hyperthermophilic archaeobacterium *Pyrococcus woesei*:
 1135 characterization of the enzyme, cloning and sequencing of the gene, and expression in
 1136 *Escherichia coli*. *J. Bacteriol.* 172, 4329–4338.

Table 1 **Macronutrients (adapted from Kayhanian and Rich (1995))**

Macronutrients		
Element	Roles	Reference
C	Carbon is a basic building block of bacterial cell material. If a carbon-containing compound is oxidized or reduced, the reaction generates energy. Because organic substrates are carbon-rich, carbon will generally not be a limiting nutrient.	(Takashima et al., 1990; Wang et al., 1979)
N	Nitrogen is the primary nutrient required for microbial synthesis of proteins.	(Takashima et al., 1990; Wang et al., 1979)
P	Phosphorus is amongst others involved in the synthesis of nucleic acids and of the energy carrier ADP/ATP	(Takashima et al., 1990; Wang et al., 1979)
K	Potassium is involved in the active transport of compounds across the bacterial membrane, e.g. the transport of nutrients and providing cation balancing.	(Takashima et al., 1990; Wang et al., 1979)
Na	Counter ions to cellular buffers, electrolytes and DNA, solutes for exchange and transport processes, energy generation	(Maret and Wedd, 2014)
S	Building block of cell materials, amino acids, and of numerous enzymes	(Bryant et al., 1971; Speece and McCarty, 1964; Wolfe, 1971; Zehnder and Wuhrmann, 1977)
Ca	Required for stability of methyltransferase and aggregation of cells, also required for stability and activity of cellulases, amylases, lipases and proteases	(Bertini and Luchinat, 1994; Brahmachari et al., 2017; Gupta et al., 2004; Holmes and Matthews, 1981; Latt et al., 1969; Lytle et al., 2000; Jisha et al., 2013; Rao et al., 1998; Schwarz, 2001; Van der Meijden et al., 1984)
Mg	Required for stability of methyltransferase, stimulate methyl-CoM reductase, methenyl-H ₄ MPT cyclohydrolase	(Inatomi, 1986; Kenealy and Zeikus, 1982; Nelson and Ferry, 1984)

Table 2 Micronutrients: elements in enzymes (adapted from Zandvoort et al. (2006))

Enzyme	Element	References
Superoxide dismutase	Cu, Fe, Zn, Mn	(Adams et al., 1986; Hughes and Poole, 1991; Kirby et al., 1981)
Hydrogenase (facultative anaerobes)	Cu	(Adams et al., 1986; Patel et al., 1993)
NO _x reductase	Cu, Fe, Mo	(Ferguson, 1994; Schindelin et al., 1997)
Ammoniummonooxygenase	Cu	(Ensign et al., 1993)
Acetyl-CoA synthase	Cu, Ni, Fe	(Hausinger, 1987; Mulrooney and Hausinger, 2003; Seravalli et al., 2003; Thauer et al., 1980)
CO-dehydrogenase	Ni, Co, Fe	(Ferry, 1999; Schönheit et al., 1979b)
Methyl-CoM reductase (F ₄₃₀)	Ni, Zn	(Hausinger, 1994, 1987; Sauer and Thauer, 2000)
Urease	Ni	(Hausinger, 1994, 1986)
Stabilize DNA, RNA	Ni	(Hausinger, 1987; Thauer et al., 1980)
B ₁₂ -enzymes, vitamin B ₁₂	Co	(Beveridge and Doyle, 1989; Hendlin and Ruger, 1950)
present in corrinoids	Co	(Schönheit et al., 1979b)
Tetrachloroethene reductive dehalogenase	Co, Fe	(Neumann et al., 1996)
Methyltransferase	Co, Mn	(Beveridge and Doyle, 1989; Fisher et al., 1973; Perry and Silver, 1982)
Hydrogenase	Fe, Se, Zn, Ni	(Albracht, 1994; Brock, T. D. et al., 1984; Fauque et al., 1988; Hausinger, 1987; Jones and Stadtman, 1977; Patel et al., 1993; Sawers, 1994; Stadtman, 1980; Takashima et al., 1990)
Glycin reductase	Se	(Heider and Bock, 1993)
Formate dehydrogenase	Se, Fe, Zn, Mo, W	(Adams et al., 1986; Brock, T. D. et al., 1984; GÅ-rio et al., 1992; Kirby et al., 1981; Sawers, 1994; Schauer and Ferry, 1982)
Formylmethanofuran-dehydrogenase	W or Mo	(Bertram and Thauer, 1994; GÅ-rio et al., 1992; Zellner and Winter, 1987)
Aldehyde-oxydoreductase, essential in acetogenic Clostridia	W or Mo	(White and Simon, 1992)
Essential in cytochrome	Fe	(Neumann et al., 1996)
Methane monooxygenase	Fe	(Lipscomb, 1994)
Nitrogenase	Fe	(Schindelin et al., 1997)
Proteases	Zi, Co, Mn	(Bertini and Luchinat, 1994; Holmes and Matthews, 1981; Latt et al., 1969; Jisha et al., 2013; Rao et al., 1998)

1141

Table 3. General mesophilic and thermophilic biomass yields for the different anaerobic phases

Phase	Mesophilic conditions		Thermophilic conditions	
	Yield (gVSS/g COD _{bio})	Reference	Yield (gVSS/g COD _{bio})	Reference
Hydrolysis and Acidogenic	≈0.15-0.18	(de Kok et al., 2013; Henze and Harremoës, 1983; Karadag and Puhakka, 2010; Silley and Armstrong, 1984; Wiegant, 1986)	≈0.1	(Oh et al., 2004)
Acetogenic and methanogenic	≈0.03-0.04	(Henze and Harremoës, 1983; Takashima and Speece, 1989; Zoetemeyer et al., 1982)	0.02-0.05	(Ahring and Westermann, 1985a; Clarens and Molleta, 1990)
Hydrolysis, acidogenic, acetogenic and methanogenic combined	≈0.18 – 0.22	(Borja et al., 1995; Henze and Harremoës, 1983; Pavlostathis and Giraldo-Gomez, 1991; Rittmann and McCarty, 2001; Takashima et al., 2011; Wolin et al., 1963)	≈0.06-0.09	(Borja et al., 1995; Takashima et al., 2011)

1142

1143
1144

Table 4 Elemental composition biomass regarding the macronutrients from literature and proposed value to calculate required amount of macronutrients

Element	Unit	Value		References	Proposed value
		Minimum	Maximum		
C	mg/gVSS	315	374	(Scherer et al., 1983; Takashima and Speece, 1989; Tchobanoglous et al., 2003; Whitman et al., 1982)	-
N	mg/gVSS	80.8	108.8		109
P	mg/gVSS	4.3	23.8		24
K	mg/gVSS	1.1	42.5		42
Na	mg/gVSS	3	40.0		40
S	mg/gVSS	5.6	12		12
Ca	mg/gVSS	0.078	3.8		3.8
Mg	mg/gVSS	0.077	0.45		0.45

1145

1146

Table 5. Proposed trace element requirement for mesophilic acidogenic systems

Trace element	Range in literature	References	Proposed value
	$\mu\text{g/g COD}_{\text{bio-influent}}$		$\mu\text{g/g COD}_{\text{bio-influent}}$
Iron	112-167	Qiang et al., 2012; Schönheit et al., 1979a; Takashima et al., 2011	165
Cobalt	0.7-14.2	Banks et al., 2012; Qiang et al., 2012; Takashima et al., 2011	14
Nickel	2.3-4.8	Qiang et al., 2012; Takashima et al., 2011	5
Zinc	29	Takashima et al., 2011	29
Copper	-	-	5
Manganese	-	-	3.75
Molybdenum	-	-	10.5
Selenium	-	-	48
Tungsten	-	-	1.65
Boron	-	-	0.057

1147

1148

Table 6. Proposed trace element requirements for mesophilic acetogenic/methanogenic systems

Trace element	Range in literature	References	Proposed value	
	$\mu\text{g/g COD}_{\text{bio-influent}}$		$\mu\text{g/g COD}_{\text{bio-influent}}$	
Iron	21-84	Qiang et al., 2012; Scherer et al., 1983; Takashima and Speece, 1989; Weimer and Zeikus, 1978; Whitman et al., 1982; Zhang et al., 2003	84	
Cobalt	3.6-25	Florencio et al., 1994; Koster and Lettinga, 1984; Qiang et al., 2012; Scherer et al., 1983; Takashima and Speece, 1989; Weimer and Zeikus, 1978; Whitman et al., 1982; Zhang et al., 2003	5	25*
Nickel	0.9-5.4	(Scherer et al., 1983; Takashima and Speece, 1989)	5.4	
Zinc	0.7-18.9	Scherer et al., 1983; Takashima and Speece, 1989; Whitman et al., 1982; Zhang et al., 2003	19	
Copper	5-6.1	Scherer et al., 1983; Zhang et al., 2003	5	
Manganese	0.05-0.75	Scherer et al., 1983; Whitman et al., 1982	0.75	
Molybdenum	2.1	Scherer et al., 1983	2.1	
Selenium	9.6	Whitman et al., 1982	9.6	
Tungsten	0.02-0.33	Zellner and Winter, 1987	0.33	
Boron	0.0114	Whitman et al., 1982	0.0114	

* The higher value of 25 $\mu\text{g/g COD}_{\text{bio-influent}}$ should only be used in the case of direct conversion of methanol into methane (Florencio et al., 1994)

1149

1150

1151

Table 7. Proposed trace element requirement for thermophilic acidogenic systems

Trace element	Range in literature	References	Proposed value
	µg/g COD _{bio-influent}		µg/g COD _{bio-influent}
Iron	230-840	Nishio et al., 1991; Qiang et al., 2013; Takashima et al., 2011	840
Cobalt	4.1-45	Qiang et al., 2013; Takashima et al., 2011	45
Nickel	1.8-19.6	Qiang et al., 2013; Takashima et al., 2011	19.6
Zinc	144	Takashima et al., 2011	144
Copper	-	-	5
Manganese	-	-	11.25
Molybdenum	-	-	31.5
Selenium	-	-	144
Tungsten	-	-	4.95
Boron	-	-	0.057

1152

1153

Table 8. Proposed trace element requirements for thermophilic acetogenic/methanogenic systems

Trace element	Range in literature	References	Proposed value
	$\mu\text{g/g COD}_{\text{bio-influent}}$		$\mu\text{g/g COD}_{\text{bio-influent}}$
Iron	24.6-280	Duboc et al., 1995; Nishio et al., 1991; Qiang et al., 2013; Takashima et al., 2011	280
Cobalt	0.5-9	Duboc et al., 1995; Qiang et al., 2013; Schönheit et al., 1979b; Takashima et al., 2011	9
Nickel	27-29	Duboc et al., 1995; Qiang et al., 2013; Takashima et al., 2011	29
Zinc	1.8-96	Duboc et al., 1995; Takashima et al., 2011	96
Copper	-	-	5
Manganese	-	-	0.75
Molybdenum	-	-	6.3
Selenium	-	-	28.8
Tungsten	-	-	1
Boron	-	-	0.01

1154

1155 **Table 9. pKa values of citric acid, NTA and EDTA (Rivers and Umney, 2003)**

Chelating agent	pKa
<i>Citric acid</i>	3.13
	4.76
	6.4
<i>NTA</i>	1.9
	2.5
	9.8
<i>EDTA</i>	2.0
	2.7
	6.2
	10.3

1156

1157

Table 10 Inhibitory concentrations trace elements at which inhibition started and IC₅₀ values for mesophilic acidogenic cultures

Trace element	Range in literature (mg/l)		References	
	Start inhibition	IC ₅₀	Start inhibition	IC ₅₀
Iron	50- >100	-	Hubbert et al., 1958; Martinez and Church, 1970	-
Cobalt	0.6-7	-	Hubbert et al., 1958; Martinez and Church, 1970	-
Nickel	0.1-0.5	100 – 1300	Martinez and Church, 1970	(Forsberg, 1978; Li and Fang, 2007; Lin, 1993; Zheng and Yu, 2004)
Zinc	3.5-20	3.4 – 1500	Hubbert et al., 1958; Lin, 1993; Martinez and Church, 1970	(Forsberg, 1978; Li and Fang, 2007; Lin, 1993; Zheng and Yu, 2004)
Copper	0.5-1.0	0.7 – 350	Hubbert et al., 1958; Martinez and Church, 1970	(Forsberg, 1978; Li and Fang, 2007; Lin, 1993; Zheng and Yu, 2004)
Manganese	50-100	-	Martinez and Church, 1970	-
Molybdenum	500	-	Martinez and Church, 1970	-
Selenium	5	0.038 – 0.14	Martinez and Church, 1970	(Forsberg, 1978)
Boron	200	-	Martinez and Church, 1970	-

1158

1159

1160
1161

Table 11. Inhibitory concentrations trace elements at which inhibition started and IC₅₀ values for mesophilic acetogenic/methanogenic cultures

Trace element	Range in literature (mg/l)		References	
	Start inhibition	IC ₅₀	Start inhibition	IC ₅₀
Nickel	1-5	1 – 1600	Ahring and Westermann, 1985b	(Ahring and Westermann, 1985b; Altaş, 2009; Fang and Chan, 1997; Fang and Hui, 1994, 1994; Lin and Chen, 1999, 1997; Lin, 1992, 1992)
Zinc	-	7.3 - 270	-	(Altaş, 2009; Fang and Chan, 1997; Fang and Hui, 1994, 1994; Lin and Chen, 1999, 1997; Lin, 1992, 1992)
Copper	1-10	1 - 175	Ahring and Westermann, 1985b	(Ahring and Westermann, 1985b; Altaş, 2009; Fang and Chan, 1997; Fang and Hui, 1994, 1994; Lin and Chen, 1999, 1997; Lin, 1992, 1992)

1162

1163

Table 12. Typical form of addition of elements (adapted from krishna Ravuri (2013))

Element	Typical form of addition
<i>Macronutrients</i>	
Nitrogen	NH ₃ , NH ₄ Cl, NH ₄ HCO ₃
Phosphorus	NaH ₂ PO ₄
Potassium	KCl
Sodium	NaCl, NaHCO ₃
Sulfur	Na ₂ S, cysteine, -SO ₄ salt
Calcium	CaCl ₂
Magnesium	MgCl ₂ , MgSO ₄
<i>Trace elements</i>	
Iron	FeCl ₂ , FeSO ₄
Cobalt	CoCl ₂
Nickel	NiCl ₂
Zinc	ZnCl ₂ , ZnSO ₄
Copper	CuCl ₂ ·2 H ₂ O, CuSO ₄
Manganese	MnCl ₂
Molybdenum	NaMoO ₄
Selenium	Na ₂ SeO ₃
Tungsten	NaWO ₄
Boron	H ₃ BO ₃

1164