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*Original Research Article*

# THE INFLUENCE OF TRACE ELEMENTS ON ANAEROBIC DIGESTION PROCESS

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A b s t r a c t

The article is the literature review on the importance of trace elements supplementation in the methane fermentation process. The production of biogas, including methane, as well as the efficiency of the process depend on the substrates to be fermented. Substances supplied with the substrate as well as products generated in the decomposition phases can inhibit the process. The factor limiting fermentation is the rate of enzymatic hydrolysis of substrates. Certain compounds, such as alkanes, alkenes, biphenol, aromatic hydrocarbons, alcohols and ketones, are not directly susceptible to hydrolysis. They undergo this process in the presence of extracellular enzymes.

The instability of the methane fermentation process described in the literature may be related to the lack of trace elements or micronutrients. Trace elements (Co, Ni, Cu, Mn, Fe, Zn, Se and Mo) are components of enzymes, some bacterial nucleic acids and essential for the synthesis of vitamins. The role of some trace elements, eg. Fe or Mo, has been well understood, while the importance of others still needs to be clarified. Literature data indicate that supplementing trace elements not only prevents process inhibition, but can also improve its performance by providing higher methane production.

Keywords: anaerobic digestion, trace elements, process inhibition, biogas production

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## 1. INTRODUCTION

Anaerobic digestion is a multi-stage process, the main stages of which are: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The phase limiting the speed of anaerobic digestion of substrates with high solids concentration is i.a. speed of enzymatic hydrolysis of non-solvable organic polymers to solvable forms available to microorganisms [20]. Hydrolysis is a biochemical reaction with double exchange between water and solved in it substance catalysed by enzymes. The process plays major role in organic compounds transformations, where, apart from hydrolysis of esters (reversable reaction), it consists of irreversible processes, such as sugar inversion, protein decomposition or fat saponification. Certain compounds, such as alkanes, alkenes, biphenol, aromatic carbohydrates, alcohols and ketones are not susceptible to hydrolysis. Extracellular enzymes, such as hydrolases and liases enable migration of solved organic substances and matter exchange with the environment. Hydrolases catalyse hydrolysis with participation of water. The most important among them are esterases, glycosidases, proteases and lipases. Liases, on the other hand, are enzymes that reversibly or irreversibly catalyse group detachment from the substrate without participation of water. They include enzymes catalysing breaking the -C-C- bond, e.g. decarboxylases of amino acids, or others decomposing bonds of C-O, C-N, C-S types. Enzymes characterise with not only high specificity of selection of particles they influence and of products they create, but also with possibility of regulating enzymatic activity as a result of concentration change of substrate or particles called cofactors. Some cofactors are inorganic compounds or ions of trace elements**,** e.g. zinc, iron, or copper. Others are organic compounds, for example vitamins. Therefore, correct course of biochemical transformation requires, besides basic structural elements, access to macro- and microelements (iron, cobalt, molybdenum, selenium, calcium, magnesium, zinc, copper, manganese, boron, or vitamin B12) [13].

Meanwhile, the substrates decomposition degree in anaerobic conditions depends on i.a. biodegradable organic carbon content and availability of nutrients.

In anaerobic digestion, the need for biogenic substances is low due to low biomass increase. The basis of waste biodegradability assessment are dependencies, the optimal values of which for methane production are as follows [3]:

- C:N = from 10:1 to 25:1;
- C:P = 113:1;
- C:N:P:S = (500-1000):(15-20):5:3 or COD:N:P:S = 800:5:1:0.5.

The decomposition of organic matter causes release of nitrogen compounds. Deublein and Steinhauser [3] prove that ammonia of 80 mg/L concentration

hinders the process, and of 150 mg/L concentration is toxic for mesophilic bacteria of anaerobic digestion. Ammonium nitrogen acts as an inhibitor in concentration between 1500 and 10000 mg/L, and becomes toxic at any higher concentration. Nitrogen content and form ($NH_3/NH_4^+$) depend on pH of the digestive mix [21,25]. Not only the form of nitrogen is significant, but also the C:N quotient, since nitrogen compounds are built into cell structure of the bacteria [25].

Substrates with a low value of C:N quotient cause ammonium nitrogen concentration increase and methane production inhibition. High values of the C:N quotient in substrates mean low amounts of nitrogen for protein synthesis, which disrupts metabolism and energy transformation in cells. In the case of exceeding the maximum value, nitrogen is quickly used up by methanotrophic bacteria, which may decrease the amount of produced biogas [20]. If the quotient decreases below the lowest value, nitrogen gets released in form of ammonia and increases pH of the digestive mix. This in turn disrupts nitrogen balance and is toxic to methanotrophic bacteria [3,13,21,25]. Anaerobic oxygenation of ammonium nitrogen through supplementation, e.g. of Fe(III) compounds (Feammox process), plays significant role in nitrogen circulation in the environment and keeps nitrogen in the digestate, allowing it to be used as a fertilizer (Fig.1.)[2,30].

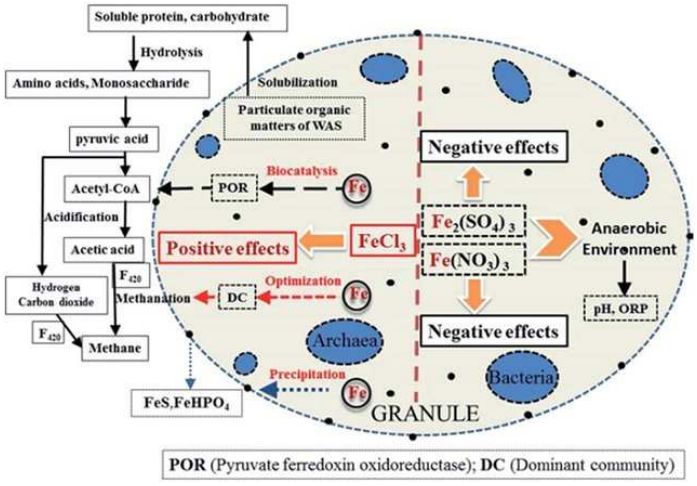


Fig. 1. Possible mechanisms of different ferric salts in the anaerobic digestion [2]

## 2. THE ROLE OF TRACE ELEMENTS IN THE ANAEROBIC DIGESTION PROCESS

Trace elements fulfil various roles in biochemical transformations and mechanisms of the anaerobic digestion process:

**Cobalt** (Co): Cobalt is present in specific enzymes and corrinoids. It is required for synthesis of vitamin B12 (cyanocbalamin) and it activates carboxylpeptidase. A corrinoid, such as vitamin B12, containing cobalt ion is known to bind to coenzyme M (CoM) methylase which catalyses methane formation in both acetoclastic methanogens and hydrogenotrophic bacteria. Co is essential for enzyme methyltransferase which catalyse the transfer of one methyl group. The common enzyme carbon monoxide dehydrogenase (CODH) uses cobalt as well. CODH plays an essential role in the acetogenic process [17,18]. Some lab tests have confirmed the positive effect of cobalt addition on methanogenesis. Kim et al. [14] reported that the supplementation of Ca, Fe, Co, and Ni to a thermophilic non-mixed reactor was required in order to achieve a high conversion of propionate at high concentrations of VFAs. Co addition to a UASB increased methane production by a factor of three, stimulating both acetogens and methanogens. On the other hand, a combined supplementation of Ni and Co was found to increase methane production from methanol conversion by methanogens [34]. Feng et al. (2010) [7] employed a laboratory-scale test using industrial food waste and municipal solid waste to demonstrate the positive effect of Co on biogas production.

**Nickel** (Ni)**:** Many anaerobic bacteria are dependent on nickel when carbon dioxide and hydrogen are the sole source of energy. The nickel tetrapyrrole, coenzyme F430, is known to bind to methyl-S-CoM reductase which catalyses methane formation from methyl-S-CoM in both acetoclastic and hydrogenotrophic methanogens. This coenzyme (F430) is contained within the Methylcoenzyme M reductase enzyme, which reduces methyl coenzyme M to methane in all methanogenic pathways [11]. In addition, CODH is a nickel protein and may aid sulphur- reducing bacteria. The role of Ni in methanogenesis is related to the following enzymes: CODH, methylreductase, hydrogenases and synthesis of F430. Supplementation of Co and Ni has been demonstrated to increase biogas production during the anaerobic digestion of organic matter such as sulfur-rich stillage, while Ni alone has been found to increase methane production to 10 g acetate $VSS^{-1}$ $d^{-1}$ [10].
Pobeheim H. et al. [22] were investigated the effect of a well-defined trace element solution and the elements nickel and cobalt on anaerobic digestion of a synthetic model substrate for maize silage was studied in batch reactor experiments at 35°C. The defined substrate consisted of xylan and starch as the

main carbon source, urea as nitrogen source and phosphorus from a 0.1 M potassium phosphate buffer. Results showed an increase of methane yield of up to 30% upon addition of the trace element solution. With an addition of nickel at 10.6 LM, a final yield of 407 L $kg^{-1}$ ODM was reached and an enhanced methane production by 25% at day 25 of operation was observed. Total elimination of nickel from the trace element solution highly decreased methane formation and process stability. Cobalt in a concentration range of 0.4 up to 2.0 LM increased the methane production by 10% approximately.

**Copper** (Cu): The role of copper in methanogenesis is subject to conflicting observations. Copper has been found in many methanogenic bacteria strains, but copper addition has not been found to have any noticeable stimulatory effects on biogas production. As the effect of this metal in methanogenesis has only been studied through being part of a trace metal mix supplementation, it is currently impossible to understand with any certainty the role of Cu in biogas production [28].

**Manganese** and **Magnesium** (Mn and Mg): Mn stabilises methyltransferase in methane-producing bacteria and acts as an electron acceptor in anaerobic respiration processes [15]. Is often interchangeable with Mg in kinase reactions. It is not clear if these metals are effectively related to biogas production. As with copper, the roles of Mg and Mn in methanogenesis have only been studied by supplementation in trace metal mixes [28]. The results showed that the stimulatory effects were in the following order: $Mn^{2+}$ >$Ni^{2+}$ >$Zn^{2+}$ >$Fe^{3+}$ >$Cu^{2+}$and the normal- and iso- HBu degradation activities of the methanogens increased by 14–25% and 17–43%, respectively [5].

**Iron** (Fe): Iron plays numerous roles in anaerobic processes, primarily due to its extremely large reduction capacity. The importance of Fe depends on its redox properties, and its engagement in energy metabolism. Fe is utilized in the transport system of the methanogenic bacteria for the conversion of $CO_2$ to $CH_4$, and functions both as an electron acceptor and donor [29]. Fe addition of 10 μM almost doubled the methanogenic activity of the sludge in a UASB reactor in the conversion of methanol [32]. It is clear that iron and nickel are present in the form of a Ni-Fe-S cluster and Fe-S cluster, and these are mainly subunits of enzymes such as hydrogenase and acetyl-CoA synthase [28]. Fe also acts as a binding component in sulfide precipitation as it is often supplemented into anaerobic reactors, not only to precipitate the formed sulfide, but also to control the level of hydrogen sulfide in the biogas [10]. In the Espinosa et al. [4] research increased the organic loading on a UASB reactor treating cane molasses stillage from 5 to 21.5 kg $CODm^{-3}$ $d^{-1}$, an accumulation of VFAs, principally propionic acid, was observed. The addition of Fe (100 mg $L^{-1}$), Ni (15 mg $L^{-1}$), Co (10 mg $L^{-1}$), and Mo (0.2 mg $L^{-1}$) reduced the level of acetic acid and

propionic acid significantly (1100 to 158mg $L^{-1}$ and 5291mg$L^{-1}$ to 251mg $L^{-1}$, respectively). After addition of Fe, Ni, and Co, the propionate and acetate utilization rates in thermophilic and mesophilic digesters increased as much as 50% and 35%, respectively [34]. The requirement of trace metals depend on different methylotrophic pathway. In general, Fe, Ni, and Zn requirements are roughly equal for $CO_2$ reduction, acetoclastic, and methylotrophic pathways [9].

**Zinc** (Zn): Although zinc is part of enzymes such as formate dehydrogenase (FDH), super dismutase (SODM), and hydrogenase, it has not yet proven to be an essential metal for methanogenesis, but Zn was found in remarkably high concentrations (50–630 ppm) in 10 methanogenic bacteria [24].

**Selenium and Tungsten** (Se and W): Selenium is a component of several anaerobic bacterial enzymes and certain bacterial nucleic acids. A common selenium enzyme in anaerobic bacteria is formate dehydrogenase (FDH). Tungsten is also a component of the FDH enzyme. W is also found in enzymes, such as formate dehydrogenase (FDH), which catalyzes formate production by propionate oxidizers and some methanogenic bacteria contain W and Mo-containing enzymes for the same purpose [2]. The W enzyme is synthesized when either W or Mo is available. If the growth medium contains Mo, the W enzyme will contain Mo rather than W [12]. Few studies have dealt with the influence of Se and W in methanogenesis. Feng et al. [7] employed a laboratory-scale reactor treating food industry waste with the aim of investigating the effects of Co, Ni, Mo, B, Se, and W on biogas production. The results showed the highest methane production was linked to addition of Se and W in combination with Co. The most important and best characterised biological form of Se is that of the amino acid selenocysteine (Sec), the 21st genetically encoded amino acid. It is structurally identical to cysteine (Cys), only with the thiol group replaced by a selenol group. The use of Sec can be partly explained by its high nucleophilicity and the fact that the selenol group is mostly deprotonated at physiological pH due to its lower *pKa* value (5.2 for Sec, 8.3 for Cys) making it more reactive than Cys. Due to this trait, Cys is almost exclusively found in the catalytic site of numerous redox-active [23]. The lack of Se in the influent to an anaerobic treatment process can result in growth limitations for some methanogens, a decrease in microbial activity, and ultimately process [11].

**Molybdenum** (Mo)*:* Molybdenum is present in the common enzyme formate dehydrogenase (FDH), which catalyzes formate production by propionate oxidizers [2]. The Mo enzyme is synthesized only when Mo is present in the growth medium [15]. However, molybdenum may also inhibit sulphate reducing bacteria, limiting the formation of necessary sulphides. Mo seems to stimulate methane production from maize silage substrate [22] and from municipal solid waste [27]. The required amounts of trace metals explained by the unique trace

metal requirement for different methanogenic pathways shows that requirements of Mo and W are higher for $CO_2$ reduction and methylotrophic pathways, whereas Co requirements are higher for the methylotrophic pathway [9].

## 3. TRACE ELEMENTS SUPPLEMENTATION

The reported concentration of trace metals required during anaerobic digestion differs significantly, depending on operating temperature (mesophilic or thermophilic), the substrate type, digestion operating mode (mono or co-digestion), and also type of methanogens, which in turn leads to a diversity of biochemical processes involved in metal dynamics. Different substrates have different metal contents also mesophilic and thermophilic anaerobic systems may have significantly different nutrient requirements. As Takashima et al. [26] reported the minimum requirements for, Ni, Co, Zn, and Fe and in thermophilic glucose fermentation to be 0.40, 0.45, 2.0, and 3.5 and mg $L^{-1}$, respectively. These required amounts are higher than those required for mesophilic acetate fermentation or mesophilic anaerobic digestion of organic solid waste [26,27].
Described in the literature instabilities of anaerobic digestion may be connected to lack of microelements or trace elements. Trace elements supplementation (Co, Ni, Cu, Mn, Fe, Zn, Se and Mo) not only prevents inhibition process, but can also improve anaerobic digestion ensure higher methane production [2,33]. The metal: Mn, Fe, Co, Cu, Mo, Ni, Se, W based on their concentration in the cells can be classified as micronutrients [16], and their concentration in the cells range from $10^{-6}$ to $10^{-15}$M [31]. It should be noted, however, that on the other hand, trace elements themselves are non-biodegradable and accumulate in biomass, constituting a potential cause of anaerobic digestion inhibition. Heavy metal elements could stay inhibition to anaerobic organisms due to the disruption of the enzyme function and structure [32]. Many previous findings have pointed out that the inhibition degree depends upon many factors, such as the total metal concentration, chemical forms of the metals, pH, and redox potential [25,33].
Trace metals can be supplemented individually, or in combination, to the anaerobic digesters. Evranos and Demirel [5] found that during the anaerobic mono-digestion of maize silage, the maximum methane yield of 0.429 L $CH_4$ $g^{-1}$ VSS added was achieved when Mo, Co, and Ni were supplemented at the same time at concentrations of 0.25, 0.5 and 0.5 mg $L^{-1}$, respectively.

## 4. BIOAVAILABILITY OF TRACE ELEMENTS

Bacteria have the ability to adjust to concentration of certain elements, thus making it very difficult to precisely determine their safe concentration. In the

case of certain inhibitors, it can be even regarded as reciprocal interaction. Different metals have different bio-uptake processes due to different kinetic and equilibrium processes. The presence of one metal can also have an impact on the speciation and thus the bioavailability of another; high Fe concentrations in an anaerobic digester can promote co-precipitation, adsorption, and ion substitution of Co and Ni on FeS [10]. Therefore, in order to reduce the amount of trace metals required and maximize the methanogenic activity, it is important to understand how their speciation affects their bioavailability.

## 5. CONCLUSIONS

The possible influence of trace elements on anaerobic digestion depends on eenvironmental conditions, their content in the substrates, and bioavailability and activity of microorganisms. Therefore, it is crucial to find a substrate/supplement which will contain trace elements in a form accessible for microorganisms.

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## WPŁYW PIERWIASTKÓW ŚLADOWYCH NA PROCES FERMENTACJI METANOWEJ

S t r e s z c z e n i e

Artykuł stanowi przegląd literatury dotyczący znaczenia pierwiastków śladowych w procesie fermentacji metanowej. Produkcja biogazu w tym metanu, jak również efektywność procesu zależą od substratów poddawanych fermentacji. Substancje dostarczane z substratem jak i produkty powstające w fazach rozkładu mogą hamować proces. Czynnikiem limitującym fermentację jest szybkość enzymatycznej hydrolizy substratów. Niektóre związki, takie jak alkany, alkeny, bifenol, węglowodory aromatyczne, alkohole i ketony, nie są bezpośrednio podatne na hydrolizę. Ulegają temu procesowi w obecności enzymów zewnątrzkomórkowych.

Opisane w literaturze niestabilności przebiegu procesu fermentacji metanowej mogą być związane z brakiem mikroelementów lub pierwiastków śladowych. Pierwiastki śladowe (Co, Ni, Cu, Mn, Fe, Zn, Se i Mo) są składnikami enzymów, niektórych bakteryjnych kwasów nukleinowych i niezbędne do syntezy witamin. Rola niektórych pierwiastków śladowych np. Fe czy Mo, została dobrze poznana, podczas gdy znaczenie innych nadal wymaga wyjaśnienia. Dane literaturowe donoszą, że uzupełnianie pierwiastków śladowych nie tylko zapobiega inhibicji procesu, ale może również poprawić jego wydajność zapewniając wyższą produkcję metanu.

Słowa kluczowe: fermentacja metanowa, pierwiastki śladowe, inhibicja procesu, produkcja biogazu

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