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Biodegradability during Anaerobic Fermentation Process Impacted by Heavy Metals

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Abstract

In the past decades, biotechnologies for reutilizing the biomass harvested from the metal-contaminated land draw attention to many scientists. Among those technologies, anaerobic fermentation is proven as an efficient conversion process for biowaste reduction with simultaneous recovery of biogas as an energy source. During the process of anaerobic fermentation, the release of metals from the biomass will impact the growth and performance of microorganisms in reactors, which then results the variation of substrate degradation. In this chapter, the impact of metals on the degradation of substrate at different stages of fermentation process, as indicated by variations of lignocelluloses, chemical oxygen demands (COD), volatile fatty acids (VFAs), etc., will be summarized. The objective is to rationalize the relationship between metal presence and substrate degradability and give suggestions for future research on metal-contaminated biomass reutilization.

Keywords: anaerobic fermentation, heavy metal, biodegradation, lignocelluloses, chemical oxygen demands, volatile fatty acid

1. Introduction

The rapid development of industries such as electronic, mining, agrochemical, tannery, and battery industries has led to an increase in the direct and indirect discharge of metals into the environment. Some metals are potentially toxic, and unlike some organic contaminants, they are not biodegradable; thus, they may accumulate in terrestrial and aquatic organisms [1]. Hence, removal of potentially toxic metals (PTM) from contaminated environments has become an issue of urgent concern. In recent years, phytoremediation, which is defined as the use of plants to remove contaminants from contaminated environment, has drawn great attention probably because it is cost-effective and sustainable [2, 3]. However, disposing the biomass residues following phytoremediation is challenging [4–6]. Therefore, there has been a growing interest on the development of inexpensive disposal techniques and improvements in bio-resource utilization to foster sustainability in remediation systems [7].

Anaerobic fermentation is a relatively efficient conversion process for biomass waste reduction with simultaneous recovery of biogas as an energy source [8–11]. In an anaerobic reactor, there are four processes that occur simultaneously, i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis [12]. Hydrolysis

process involves the conversion of macromolecules such as proteins, polysaccharides, and fats that compose the cellular mass of the excess sludge into water-soluble molecules with a relatively small molecule (e.g., peptides, saccharides, and fatty acids) [12]. Simple molecules with a low molecular weight such as volatile fatty acids (e.g., acetic, propionic, and butyric acid), alcohols, aldehydes, and gases like CO₂, H₂, and NH₃ are produced via acidification of the hydrolyzed products (acidogenesis) [12]. The acidification products are converted into acetic acids, H₂ and CO₂, by acetogenic bacteria in a process called acetogenesis. These first three steps of anaerobic digestion are often called acid fermentation, and they help transform the waste biomass into substrates for methanogenesis [12]. In the methanogenesis process, the products of the acid fermentation (mainly acetic acid) are converted into CO₂ and CH₄.

Microorganisms responsible for anaerobic fermentation require a trace amount of metals (e.g., Ni, Co, Cu, Fe, Zn, etc.) for their optimum growth and performance [13]. Various enzymes involved in anaerobic metabolism use trace metals as their cofactors. For example, methanogenic enzymes such as CO dehydrogenase (CODH) and methyl-H₄MPT:HS-CoM methyltransferase use cobalt acts as their cofactor [14].

However, waste biomass often contains varying amounts of metals depending on the source of the biomass. During the process of anaerobic fermentation, the release of metals from the biomass will influence the efficiency of fermentation by affecting the enzyme activity, microorganism community, and even degradation and metabolic pathways [15]. In this chapter, the impact of metals on the degradation of substrate, as indicated by variations of lignocelluloses, chemical oxygen demands, volatile fatty acids, etc., will be summarized. The objective is to rationalize the relationship between metal presence and substrate degradability during different fermentation stages and give suggestions for future research on metal-contaminated biomass reutilization.

2. Hydrolysis stage

Hydrolysis is oftentimes the rate-limiting step in the anaerobic digestion process probably because fermentative bacteria require an additional step of excreting extracellular enzymes, such as cellulases and lipases, to carry out the hydrolysis or solubilization process [16, 17]. It can be accelerated by enhancing the accessibility of anaerobic microorganisms to intracellular matter or cellulose using thermal, chemical, biological, and mechanical processes, as well as their combinations [18].

2.1 Lignocellulose degradation

Lignocelluloses are mainly composed of cellulose, hemicellulose, and lignin [19]. The cellulose and hemicellulose themselves are relatively easy to be broken down by microorganisms; however, their biodegradability decreases when they occur in lignocellulose complexes [20]. The impacts of metals on lignocellulose degradation vary with the metal species, concentrations, and fermentation conditions.

Previous studies showed that the presence of metals at certain concentrations may enhance the degradation of lignocelluloses [21, 22]. In one study, an average lignocellulose content of $87.49 \pm 3.19\%$ TS was obtained in a control group but decreased to $80.44 \pm 3.41\%$ TS, $77.94 \pm 3.50\%$ TS, and $79.45 \pm 2.88\%$ TS following the addition of 30, 100, and 500 mg/L Cu and $79.36 \pm 3.72\%$ TS, $79.10 \pm 2.80\%$ TS, and $76.60 \pm 2.97\%$ TS following the addition of 30, 100, and 500 mg/L Cr, respectively. Thus, Cu and Cr addition significantly enhanced the degradation of lignocellulose [21, 22].

Several studies on methanogenic bacteria found Cu, suggesting it could be a critical component for the enzymes super dismutase and hydrogenase [23]. However, at relatively high concentrations, Cu can inhibit anaerobic fermentation, which results in reduction of degradation efficiency. Cu changes the physiological steady state of the fermentation process by inhibiting the degradation of the substrate and the growth of the microbes [8, 24]. In contrast, Cu has been shown to enhance the biogas production via fermentation [9, 25]. Despite the inhibitory effects of Cu, biogas production was probably enhanced by the addition of sulfide to the digester in stoichiometrically equivalent amounts [26]. Our research suggested that the promoting effect of Cu addition on biogas yields was mainly attributable to better process stability, the enhanced degradation of lignin and hemicellulose, the transformation of intermediates into VFA, and the generation of CH₄ from VFA [22].

Cr is one of the heavy metals that have often been blamed for unsatisfactory operation or failure of anaerobic digesters [27]. Contradictory toxicity levels of Cr on anaerobic fermentation have been cited in literatures [28, 29]. This is probably because of differences in availability of Cr in fermenters (which is influenced by the precipitation and adsorption of soluble metals), differences in materials used in the studies [30], and dissimilar operational conditions (e.g., temperature, pH, hydraulic retention time, solid retention time, and mixed liquor volatile suspended solids) [27]. Cr in certain concentrations was found to promote the efficient generation of CH₄ by inducing better process stability, enhancing degradation of lignin and hemicellulose, transforming intermediates into VFA, and increasing coenzyme F₄₂₀ activities [21].

According to our recent study, when compound metals were added into the fermentation reactors, the degradation of lignocelluloses performed differently (**Table 1**). It was found that the addition of Zn into the Cd- or Cu-containing reactors enhanced the degradation of lignin and cellulose significantly which resulted in a significant decrease in the total lignocellulose contents. The addition of Fe together with Cd reduced the cellulose contents and the total lignocellulose contents. In contrast, the addition of Ni into either Cd- or Cu-containing reactors did not improve the degradability of the feedstocks.

Depending on the methanogenic pathway, the general trends of metal requirements are as follows: Fe is the most abundant metal, followed by Ni and Co and smaller amounts of Mo (and/or W) and Zn [31]. Almost all metalloenzymes involved in the pathway of biogas production contain multiple Fe₂S₂, Fe₃S₄, or Fe₄S₄ clusters [17, 31, 32]. Fe is primarily present as Fe-S clusters used for electron transport and/or catalysis, as well as attenuating disturbances associated with the presence of sulfide which often results in a more stable process [31, 33–35]. Zn, like Cu, is present in relatively large concentrations in many methanogens. Zn is important in anaerobic fermentation because it is required by enzymes involved in methanogenesis such as coenzyme M methyltransferase [36]. At certain concentrations, Zn can promote biogas production [37, 38]. For example, during the swine manure anaerobic digestion, Zn concentrations in the range of 125–1250 mg/L improved significantly microbial activity [39].

Ni is an important trace element for many prokaryotic microorganisms that are in the *Bacteria* and *Archaea* domains [40]. It is required in the prosthetic groups of a total of eight enzymes that are found in prokaryotic microorganisms, including CODH, acetyl-CoA synthase/decarbonylase, methyl-coenzyme M reductase (MCR), [NiFe]-hydrogenases, superoxide dismutase (Ni-SOD), glyoxylase I, urease, and acireductone dioxygenase [41]. Generally, the biologically relevant oxidation states of Ni are Ni⁺, Ni²⁺, and Ni³⁺, and these depend on how Ni is ligated to the protein. Ni usually functions either as a redox catalyst, for example, as in the case of hydrogenase or CODH where Ni is liganded by cysteinyl sulfurs [40]. However, Ni

Metals concentrations (mg/L)	Lignin (%TS)	Hemicellulose (%TS)	Cellulose (%TS)	Total lignocellulose (%TS)
Cd(1.0)	19.84 ± 0.94	13.14 ± 0.75	19.27 ± 1.38	52.25 ± 3.07
Cd(1.0) + Fe(10.0)	18.20 ± 0.63	11.96 ± 0.61	15.97 ± 0.90*	46.13 ± 2.14*
Cd(1.0) + Ni(2.0)	20.82 ± 1.10	15.02 ± 0.75	16.36 ± 0.60	52.20 ± 2.45
Cd(1.0) + Zn(2.0)	12.83 ± 1.07**	12.98 ± 0.64	13.55 ± 1.13**	39.36 ± 2.84**
Cu(1.0)	19.63 ± 0.85	13.23 ± 0.75	19.34 ± 1.46	52.21 ± 3.06
Cu(1.0) + Fe(10.0)	16.92 ± 0.90	11.44 ± 0.61	16.46 ± 0.83	44.83 ± 2.34**
Cu(1.0) + Ni(2.0)	19.95 ± 1.15	12.05 ± 0.69	20.21 ± 0.74	52.21 ± 2.58
Cu(1.0) + Zn(2.0)	14.56 ± 1.03**	12.34 ± 0.61	14.43 ± 1.18**	41.34 ± 2.82**

Mean ± standard error. n = 10.

*p < 0.05.

**p < 0.01.

Table 1.

The average contents of cellulose, hemicellulose, and lignin and total lignocellulose during the anaerobic co-digestion of corn stover and cow dung (55.0 ± 1.0°C) in the presence of different compound metals.

can act synergistically in Ni–Cu, Ni–Mo–Co, and Ni–Hg systems or antagonistically in Ni–Cd and Ni–Zn systems [42]. Ni was also found to decrease the toxicity of Cd and Cu [43]. However, the combination of Ni and Cd or Cu has been shown that they do not promote the degradation of lignocelluloses (Table 1). However, this line of inquiry requires further study.

2.2 Variation of chemical oxygen demands (COD)

The soluble organic components in the fermenter, shown as COD, originate from the hydrolysis process that liquefies large molecules; long-chain natural polymers of the substrate-like cellulose, hemicellulose, lignin, and polysaccharides; and proteins by extracellular enzymes [17, 44]. Previous studies on anaerobic fermentation of crops and manure showed that the COD in the reactor increased and then decreased because organic matter in the liquid was generated first and then consumed to produce the biogas. Therefore, greater COD did not cause greater biogas generation [45].

Previous studies demonstrated that COD initially increased and then decreased in the presence of Cu [22]. During the initial stage of the fermentation, the substrate was rapidly hydrolyzed into small organic molecules, bringing about an increase of COD in the first 5 days. Later, the COD of the Cu-added groups decreased. The COD of the control group decreased more slowly than those of the Cu-added groups, and the discrepancy between them increased during the fermentation. Taking the whole fermentation process into account, the COD in the Cu addition groups were relatively lower than the control group. It was suggested that Cu addition enhanced the utilization of organic molecules in the fermentation (as indicated by the decrease of COD) and the biogas production [22]. A similar promoting effect was found in the Cr-stressed anaerobic fermentation process [21]. However, Cr addition did not yield lower COD than the control group.

The COD were generally lower in Fe-added groups than in the control group [46]. Fe addition induced a stable and excellent COD conversion rate suggesting a more efficient utilization of soluble organic components in the fermenter that consequently improves biogas yields [47]. Likewise, Ni addition influenced the biogas production, and this can also be partly explained by the Ni effect on COD [48].

Treatments	Parameters	55.0 ± 1.0°C	45.0 ± 1.0°C	35.0 ± 1.0°C	25.0 ± 1.0°C
No Cd added	COD (mg/L)	10172.01 ± 1246.81	11219.30 ± 1596.13	12116.95 ± 2317.61	9517.14 ± 1199.70
	Biogas yield (mL/g TS)	67.65 ± 1.16	58.45 ± 1.01	19.42 ± 0.32	15.96 ± 0.27
Cd added	COD(mg/L)	9651.83 ± 1505.46	11052.13 ± 1612.40	13192.66 ± 2518.87	9829.71 ± 398.46
	Biogas yield (mL/g TS)	341.62 ± 5.88	68.35 ± 1.18	20.51 ± 0.34	15.84 ± 0.27

Table 2. The average COD and cumulative biogas yields during the anaerobic co-digestion of acid-pretreated corn stover and cow dung (55.0, 45.0, 35.0, and 25.0 ± 1.0°C) with and without Cd (1.0 mg/L) addition. Mean ± standard error.

Lower COD concentrations and higher biogas yields were obtained using higher Ni concentrations. The results demonstrated the balance of different fermentation steps (from hydrolysis to methanogenic phase). Moreover, as the substrates in the Ni-added groups were better degraded in the former three stages (from 4th to 13th day), the left substrates were few, and hence the COD concentrations of Ni-added groups were not increased at the end of the experiments.

The variation of COD in the presence of metals should also be considered under different fermentation temperatures. The required amounts for Ni, Co, Zn, and Fe and in thermophilic glucose fermentation were 10 times more than those required for mesophilic acetate fermentation [49, 50]. In our anaerobic fermentation experiment with acid-pretreated corn stover mixed with fresh cow dung as feedstocks, the COD at 55, 45, 35, and 25 ± 1.0°C were analyzed with and without Cd (1.0 mg/L) addition. The cumulative biogas yields and average COD during the entire fermentation process in Cd-added and no Cd-added group are shown in **Table 2**. It was found that Cd addition resulted in higher biogas yields with the increase of temperature together with the lower COD. Overall, the biogas production should be explained by the variation and/or consumption of the COD concentrations along with VFAs during the fermentation process, rather than the values of COD concentrations.

3. Acidogenesis stage

Acidification is affected by a very diverse group of bacteria, the majority of which are strictly anaerobic. As the acidogenesis stage progresses, the acidic components, including long-chain fatty acids (LCFAs), volatile fatty acids (VFAs), etc., are generated, and they cause a change in the pH. Furthermore, during acidogenesis, organic nitrogen is converted into ammonia [51].

3.1 Variation of pH values

The optimal pH range for efficient methanogenesis ranges from 6.7 to 7.4 [52]. However, the acidogenic bacteria can metabolize organic material down to a pH of around 4. At the beginning of anaerobic fermentation, the pH values are likely to decrease due to the generation of acid components. Thus, buffer solution is suggested for preventing the dramatic pH reduction.

On the one hand, the effect of metal toxicity depends on pH [53]. In general, at high pHs metals have a tendency to form insoluble metal phosphates and carbonates [54], whereas at low pHs the initial leaching of metals from the sludge occurs and hence their solubility increases [55, 56]. Soluble levels of Ni ion were found the highest, while those of Pb ions were found the least as compared to other four heavy metals from pH 4 to 12. At extreme pH of 1, Zn, Pb, and Cd ions showed higher levels than those of Ni, Cu, and Cr. However, Cu, Ni, and Zn ion levels were found higher than those of Pb, Cd, and Cr at an extreme pH of 13. Metal ion levels showed the order of Ni > Cu > Cr > Zn > Cd > Pb between pH 8 and 12. In other pH ranges, metal ions varied with pH [57].

On the other hand, the presence of metals in the reactor during the fermentation process can modify pH values. Previous studies found that adding Cu and Cr resulted in a decrease in pH at the beginning of the experiment, but the pH later recovered [21, 22]. The average pH following addition of Cu have been shown to be generally higher than control groups as well as groups in which Cr is added. An investigation on anaerobic digestion of sewage sludge found that pH negatively related with the exchangeable (−0.838, $p < 0.01$) and residual fractions (−0.753,

$p < 0.01$) of Cu while positively related to Fe-Mn oxide-bound (0.895, $p < 0.01$) and organic-bound (0.698, $p < 0.05$) fractions of Cu [58]. In contrast, pH positively related to carbonate-bound Cr (0.768, $p < 0.01$) and organic-bound Cr (0.908, $p < 0.01$) while negatively related to Fe-Mn oxide-bound Cr (-0.899 , $p < 0.01$) [58]. The results suggest the decrease of pH at the beginning of fermentation was probably beneficial for generating both the exchangeable and residual fractions of Cu. The increase of pH after the start-up of the fermentation is probably helpful for partitioning Cr to yield carbonate-bound and organic-bound fractions, thus reducing the bioavailability and toxicity of Cr [21].

Addition of Fe and Ni has been shown promote an alkalescent environment for anaerobic fermentation. Previous studies found that adding 10.0 mg/L Fe into the fermenter resulted in lower pH values ($p < 0.05$) when fermentation is around its peak stage [46]. However, following fermentation peak stage, no significant change in pH has been reported even after increasing the Fe concentration from 0.5 to 5.0 mg/L or Ni concentrations from 0.2 to 2.0 mg/L [46, 48].

3.2 Variation of NH_4^+ -N concentrations

Total ammonia (TAN), consisting of ammonium ions (NH_4^+) and free ammonia (FAN, NH_3), is produced during anaerobic degradation of proteins, urea, and nucleic acids [59]. At NH_4^+ -N concentrations below 200 mg/L, TAN is an important nutrient for microorganism growth [60]. However, it was reported by Math-Alvarez et al. [61] and confirmed in a critical review by Chen et al. [42] that NH_4^+ -N concentrations ranging from 0.6 to 14 g/L inhibited the methanogenic activity depending on different experimental conditions [62].

Previous studies showed that Cu or Cr addition induced remarkable differences in NH_4^+ -N concentrations compared to a control group [21, 22]. The NH_4^+ -N values in the control group fluctuated in the range 9.70–157.34 mg/L before the 21st day of fermentation; yet in Cu- and Cr-added groups, the NH_4^+ -N values were relatively stable with concentrations ranging from 55.98 to 113.82 mg/L [22] and 39.85 to 105.87 mg/L [21], respectively. Thus, Cu and Cr addition contributed to the stability of the fermentation system.

Table 3 shows that further addition of other metals may increase the NH_4^+ -N concentrations in the metal-stressed fermenters. According to our research, Zn addition significantly enhanced the generation of NH_4^+ -N in both Cd and Cu

Metals concentrations (mg/L)	NH_4^+ -N	Total VFAs
Cd(1.0)	558.39 ± 39.25	910.57 ± 273.75
Cd(1.0) + Fe(10.0)	604.50 ± 37.34	1865.18 ± 684.94
Cd(1.0) + Ni(2.0)	747.13 ± 38.21**	1003.57 ± 219.79
Cd(1.0) + Zn(2.0)	675.34 ± 36.22*	513.86 ± 195.52
Cu(1.0)	476.63 ± 37.36	369.77 ± 73.28
Cu(1.0) + Fe(10.0)	652.83 ± 61.88*	1562.24 ± 577.63*
Cu(1.0) + Ni(2.0)	569.31 ± 23.62	1029.20 ± 298.17
Cu(1.0) + Zn(2.0)	671.82 ± 43.40**	804.66 ± 286.14

Mean ± standard error; $n = 10$.

* $p < 0.05$.

** $p < 0.01$.

Table 3.
 The NH_4^+ -N and total VFA concentrations during the anaerobic co-digestion of corn stover and cow dung ($55.0 \pm 1.0^\circ\text{C}$) in the presence of different compound metals.

contained fermenters. Addition of Ni induced higher NH_4^+ -N concentrations in Cd-stressed anaerobic fermentation process, while Fe had a similar response in Cu-stressed fermentation processes. The results suggest that metal mixtures benefited from the degradation of substrate containing nitrogen, such as proteins (**Table 3**) together with the degradation of lignocelluloses (**Table 1**).

3.3 Variation of long-chain fatty acids (LCFAs)

Long-chain fatty acids (LCFAs) are the intermediate products of lipids' hydrolysis and thus are abundant in lipid-rich substrates such as slaughterhouse wastewater and dairy industrial sludge [63, 64]. LCFAs (e.g., oleic acid) are often degraded through β -oxidation [65] to form acetate, hydrogen, and short-chain fatty acids (SCFAs). Short-chain fatty acids are further catabolized to acetate and hydrogen following cycles of β -oxidation [66]. LCFAs can inhibit the activities of the microorganisms involved in all the AD steps [67] by attaching to bacterial cell membrane, thus limiting mass transfer [68]. It was reported that LCFAs concentration of 0.2 g/L oleate had a profound inhibitory effect, while biogas production ceased when the concentration was increased to 0.5 g/L [69]. Hwu et al. [70] reported 50% inhibition of methanogenesis in batch reactors at 0.1–0.9 g L⁻¹ oleate, depending on the origin of the bacterial inoculum. It has been previously documented that LCFAs could inhibit the activity of hydrolytic, acidogenic, and acetogenic bacteria and methanogenic archaea [68, 69, 71]. However, many studies have reported an adaptation of the microbial communities during the degradation of LCFAs [70, 72]. Moreover, the archaeal community was found to be more tolerant to increased LCFA concentration levels compared to the bacterial community [73].

Metals play a major role in several metabolic pathways and thus will impact of transformation of LCFAs during fermentation process. In general, adding adequate concentrations of microelements may accelerate the degradation of short-chain fatty acids (SCFAs) and LCFAs and would be beneficial for the anaerobic mono-digestion of food waste [74]. However, there is lack of studies on the responses of LCFAs to metal stress. Further studies are necessary for revealing the underlying mechanisms.

3.4 Variation of total volatile fatty acids

VFAs are the intermediary products of the anaerobic fermentation and a precursor for methanogenesis. The concentration of VFA is an important index to evaluate the efficiency of hydrolysis, acidification, and methanogenesis [75]. Trace metal supplementation is one method to increase VFA utilization [76].

Many studies worked on the impacts of heavy metals on the degradation of VFAs [77–79]. At the beginning of the fermentation, the total VFAs often increase due to hydrolysis of substrate and the accumulation of acidic hydrolytic products [21, 22], together with the decrease of pH values. It was reported that a pH range of 5.7–6.0 was recommended as optimal to produce VFAs [80]. During this period, high concentration of Cu was found to inhibit the acidification process [22], while high concentration of Cr inhibited the methanogenesis [21], resulting in low biogas yields. Later on, the VFAs were shown to be consumed during the biogas production, and supplementing metals greatly benefited the process [21, 22].

Supplementing metals may promote the degradation of VFAs, while a metal deficiency may result in the accumulation of VFAs, which often inhibits the anaerobic processes. For example, a previous study found Fe and Ni deficiency during anaerobic digestion of wheat stillage resulted in a rapid accumulation of VFAs [81]. In another study, excluding Co, Zn, and Ni from the methanol-based feed of an

UASB reactor induced lower specific methanogenic activity (SMA) and the accumulation of VFAs [82–84].

A strong relationship has been previously reported between VFAs and different forms of metals [58]. For example, there was a strong correlation between the organic-bound Cr and VFAs ($r = -0.846$, $p < 0.01$), indicating that decrease in VFA enhanced the transformation of Cr from unstable species to organic-bound fractions, thus reducing Cr bioavailability and toxicity [58]. As a result, there was an improvement in the CH_4 yield. However, the relationships between VFA and metals have been shown to depend on the form and species of the metal. For combining metals, the addition of Fe into Cu-contained fermenters significantly increased the total VFA concentrations (**Table 3**) and resulted in higher biogas yields (data not shown).

4. Acetogenesis stage

Many factors, including substrate concentration, hydraulic retention time, temperature, pH, and process configuration, affect the performance of the acidogenesis phase [31, 85]. However, these factors are particularly susceptible to the presence and subsequent interactions with heavy metal ions [86].

The C_2 - C_7 organic acids are predominant intermediates in the anaerobic digestion of organic matter. The anaerobic oxidation of the C_3 - C_7 substrates is coupled to a reduction of protons (H_2 formation), and the oxidation of C_3 and C_4 organic acids is thermodynamically unfavorable (endergonic process) under standard conditions [77].

Lin studied the effects of Cr, Cd, Pb, Cu, Zn, and Ni on VFA degradation in anaerobic digestion by using serum bottle assays with acetic acid acclimated seed sludge (AASS) and mixed acid acclimated seed sludge (MASS) [87]. The relative toxicity of heavy metals to degradation of acetic acid (HAc), propionic acid (HPr), and n-butyric acid (n-HBu) was $\text{Cd} > \text{Cu} > \text{Cr} > \text{Zn} > \text{Pb} > \text{Ni}$, $\text{Cd} > \text{Cu} > \text{Zn} \cong \text{Cr} > \text{Pb} > \text{Ni}$, and $\text{Cd} > \text{Cu} > \text{Cr} > \text{Zn} > \text{Pb} > \text{Ni}$, respectively [87]. Cd and Cu were the most, and Pb and Ni were the least toxic heavy metals to VFA-degrading organisms. To some heavy metals, VFA-degrading acetogens were more sensitive than HAc-utilizing methanogens. The order of sensitivity of the VFA degradation to the metallic inhibition was $\text{HPr} > \text{HAc} \cong \text{HBu}$ for Cr, $\text{HAc} > \text{HPr} \cong \text{HBu}$ for Cd and Pb, $\text{HPr} > \text{HAc} > \text{HBu}$ for Zn, $\text{HAc} \cong \text{HPr} \cong \text{HBu}$ for Cu, and $\text{HAc} > \text{HPr} > \text{HBu}$ for Ni. Mixtures of the heavy metals caused synergistic inhibition on HAc degradation [87].

Lin et al. carried out a systematic study on the effect of trace metal supplementation on anaerobic degradation of butyric acid [88]. The results showed that the stimulatory effects were in the following order: $\text{Cu}^{2+} < \text{Fe}^{3+} < \text{Zn}^{2+} < \text{Ni}^{2+} < \text{Mn}^{2+}$ and the normal and isoHBu degradation activities of the methanogens increased by 14–25% and 17–43%, respectively [88]. Kim et al. 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 [89].

About 70% of CH_4 is generated from acetic acid [86]. The acetate utilization rates required per gram of VSS are used to estimate the nutrient supplementation required to prevent limitations in methanogenic activity [90]. Addition of Fe was found to have a stimulatory effect on acetate utilization by methanogens [76]. Bhattacharya et al. [91] found adding 20 mg/L Zn^{2+} resulted in a complete inhibition of acetate degradation due to Zn toxicity to methanogenesis. Ni sites in the acetyl-CoA decarboxylase/synthase enzyme complex have been identified. This enzyme seemed to have an important role in the conversion of acetate to CH_4 [92].

5. Methanogenesis stage

Methanogenesis is the microbial process, whereby CO_2 , acetate, or methyl compounds are converted to CH_4 in order to generate ATP through the buildup of a sodium ion or proton gradient [31]. Methanogenesis is one of the most metal-rich enzymatic pathways in biology [93]. The contents of Cu, Fe, Ni, and Zn in methanogens (including 10 species of *Methanosarcina*, *Methanococcus*, *Methanobacterium*, *Methanobrevibacter*, etc.) were determined as <10–160 ppm, 0.07–0.28%, 65–180 ppm, and 50–630 ppm, respectively [36]. The key enzyme complex in producing biogas from acetate is CODH [15, 94]. CODH cleaves the C-C and C-S bonds in the acetyl moiety of acetyl-CoA, oxidizes the carbonyl group to CO_2 , and transfers the methyl group to coenzyme M. MCR catalyzes the enzymatic reduction of methyl-coenzyme M to CH_4 in methanogenesis, which includes a Ni-containing cofactor called F_{430} [15, 31, 40]. Besides, the CODH complex is also involved in the formation of acetate by acetogens from H_2/CO_2 and methanol [95]. MCR is found exclusively in methanogenic archaea [96].

5.1 Biogas yields

The stimulatory effect of trace metal supplementation in certain ranges on anaerobic digestion has been widely reported [10, 21, 46]. Depending on the methanogenic pathway, the general trends of metal requirements are as follows: Fe is the most abundant metal, followed by Ni and Co and smaller amounts of Mo (and/or W) and Zn [31].

Low concentrations of Fe have been shown to markedly increase the conversion of acetic acid to CH_4 [97]. Fe^{2+} in concentrations of up to 20 mM has been shown to increase the conversion of acetate to CH_4 [98]. It was found that Fe^{2+} marginally stimulated biogas yield and CH_4 content at 37°C and the addition of Fe^{2+} increased VFA utilization but enhanced H_2 utilization considerably [99]. The addition of Fe resulted in a stable process, and its combination with Co contributed to higher biogas production (+9%), biogas production rates (+35%), and reduced VFA concentration while simultaneously degrading the organic fraction of municipal solid waste and slaughterhouse waste [34]. The promoting effect of Fe^{2+} addition on biogas yields of mixed *Phragmites* straw and cow dung was mainly attributed to the extension of the gas production peak stage and the improvement of cellulase activities [46].

The optimum or stimulatory concentrations of Ni for batch cultures of methanogens were reported to range between 12 mg/m³ and 5 g/m³ [92]. Pobeheim et al. observed an increase in CH_4 production of 25% at day 25 of operation following addition of 10.6 μM Ni [100]. Ni addition of 1–200 μM enhanced the methane production from anaerobic conversion of acetate by 6.30–44.6% compared with the control, respectively [101]. Furthermore, the limitation of Ni in the fermenters led to process instability and was proven to reduce biogas generation [102]. On the other hand, the addition of Ni was found to be beneficial to the methanation process. Ni had increased the ratio of $\text{CH}_4:\text{CO}_2$ [103].

Besides Fe, Ni, and Co, other trace metals like Cu, Cr, and Cd were shown to promote biogas production. Lower concentrations of Cu ($1.82 \pm 0.01 \mu\text{g/g}$ dry wt.) and Cr ($0.89 \pm 0.04 \mu\text{g/g}$ dry wt.) better served as micronutrients for methanogenic bacteria and might have enhanced the process of methanogenesis and thus CH_4 content in the product biogas [9]. Cao et al. harvested five types of plant from Cu-contaminated land, including *Phytolacca americana* L., *Zea mays* L., *Brassica*

napus L., *Elsholtzia splendens*, and *Oenothera biennis* L. and investigated the effects of Cu on anaerobic digestion of these plants. Compared to normal plants with low Cu content, the plants used in remediation with increased Cu levels (100 mg/kg) not only required a shorter anaerobic digestion time but also increased the CH₄ content in biogas [25]. 30 and 100 mg/L Cu²⁺ addition increased the cumulative biogas yields by up to 43.62 and 20.77%, respectively [22]. In another study, 30, 100, and 500 mg/L Cr⁶⁺ addition increased the cumulative biogas yields by up to 19.00, 14.85, and 7.68%, respectively, while bringing forward the daily biogas peak yield [21]. Investigations on the anaerobic fermentation of five contaminated crops showed that less than 1 mg/L of Cd in plants promoted or at least had no inhibitory effect on cumulative biogas yields [11]. Jain et al. noted that at low concentrations, Cd and Ni had a favorable effect on the rate of biogas production and its CH₄ content, but with increase in concentrations, the rate of biogas production and CH₄ content decreased [104].

5.2 Biogas compositions

Biogas is composed of CH₄, CO₂, and other trace compositions like H₂. Methanogens using H₂/CO₂ as the matrix usually contain two hydrogenases: one is a hydrogenase that uses coenzyme F₄₂₀ as the electron acceptor called coenzyme F₄₂₀-reducing hydrogenase, and the other is coenzyme F₄₂₀-nonreducing hydrogenase [105]. Hence, the presence of metals in the fermenters influences the biogas composition by impacting the pathways. For example, Cu²⁺ and Cr⁶⁺ addition stimulated biogas production and the generation of CH₄ by enhancing the activities of coenzyme F₄₂₀ and methanogenesis [21, 22].

Previously, Fe was found in acetyl-CoA synthase, CH₄ monooxygenase, NO-reductase, and nitrite reductase [15]. Fe, together with Ni, was found in hydrogenases of *Methanosarcina barkeri*, which consumes H₂ to provide electrons for the reduction of CO₂ to CH₄ [106, 107].

As we studied, the compositions of biogas varied with temperatures in the presence of heavy metals. The impacts of Cd addition on biogas compositions of anaerobic co-digestion of acid-pretreated corn and fresh cow dung under different fermentation temperatures are shown in **Figure 1**. When temperature increased, the CH₄, CO₂, and H₂ contents also increased, but N₂ contents decreased in both Cd-added and control groups. The CH₄ contents reached plateau after the fourth day in 55°C group and seventh day in 45°C group (**Figure 1A**). The increase of CO₂ contents slowed down after the fourth day in both 55 and 45°C groups (**Figure 1B**). The tendency of CH₄ and CO₂ contents in 35 and 25°C groups was not detected fully as the biogas yields were too low to be collected by gas bag. Similar contents of H₂ were observed in 45, 35, and 25°C groups while lower in 55°C group (**Figure 1C**). The N₂ contents decreased more rapidly when fermentation temperature was increased (**Figure 1D**). The results indicated that elevated temperatures accelerated the start-up of fermentation.

Cd addition improved the CH₄ contents by approximately 6% after the fourth day in 55°C group. Taking the other biogas compositions into account, it was found that Cd addition decreased the CO₂ contents in the biogas while having little influences on H₂ and N₂ contents. However, the impact of Cd on biogas compositions in other three temperature groups was not significant. Therefore, thermophilic fermentation (55°C) promoted the CH₄ generation in the presence of Cd in the present study. Low temperature hindered the production of CH₄ which agreed with a previous study that used swine manure as substrate [108].

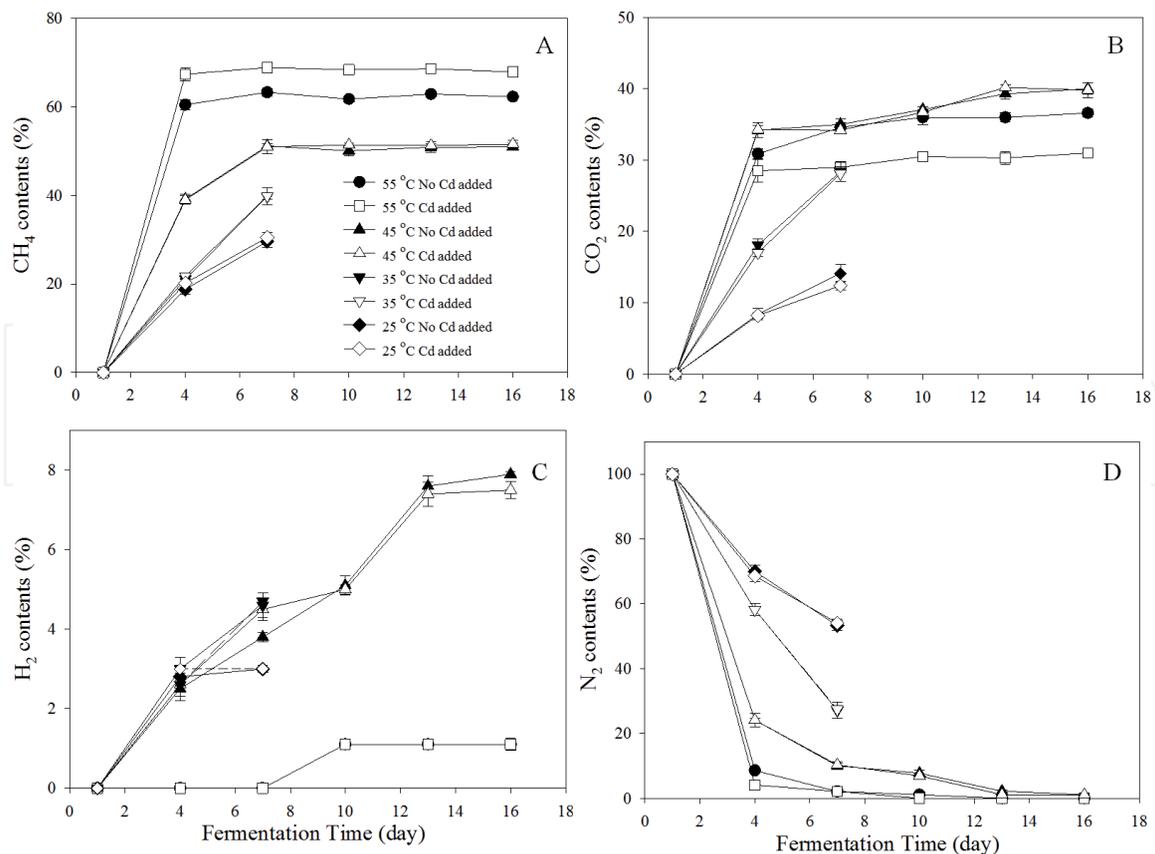


Figure 1. Biogas composition under Cd stress with fermentation temperature of 55, 45, 35, and 25°C (A) CH₄ contents, (B) CO₂ contents, (C) H₂ contents, and (D) N₂ contents.

6. Conclusions

This book chapter reviewed the past findings in the impacts of metals on different stages of the anaerobic degradation process. The requirements of metals by the enzymes involved in the anaerobic process resulted in the different performances with varied metal species and bioavailability. In general, metals in certain concentrations were able to promote the lignocellulose degradation, the generation and consumption of organic components in the fermenters like LCFAs and VFAs, the biogas production, as well as the CH₄ contents. The mechanisms of metals were studied by many scientists, focusing on the enzyme activities, microbial communities, etc.

Although a large amount of research has been carried out on individual stages or the entire anaerobic fermentation process, there are still challenges on controlling metal-stressed anaerobic degradation process for optimal utilization of metal-contaminated biowastes. Further work on bioavailability of metals during anaerobic fermentation process and detailed compositions of intermediary products, the relationships between microbial functions and metal species, etc. are recommended for better understanding of the metal-stressed anaerobic degradation process.

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Conflict of interest

No conflict of interest.

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