ROLE OF HEAVY METALS IN ANAEROBIC DIGESTION

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ABSTRACT

Stimulatory, inhibitory and antagonistic roles of heavy metals in anaerobic digestion are reviewed. Partitioning and speciation, variety of biotic and abiotic detoxifying mechanisms as well as acclimation are shown to hinder an accurate prediction of response based on a given total metal concentration.

INTRODUCTION

Metals play several important roles in anaerobic processes acting as: 1. microelements essential for various enzymatic reactions; 2. inhibitors and toxicants of the enzymatic reactions and thus the microbial biomass; 3. stimulants and promoters of bacterial aggregation; 4. inhibitors of sulfide toxicity.

Studies addressing variety of effects of metals on anaerobic processes show that numerous abiotic and process related factors interfere with the application of metal-effects data from pure cultures to industrial scale digesters (1). The net result is that precipitation, complexation, acclimation and population changes allow the digesters to operate at significantly higher (than presumed toxic level) total metal concentration or conversely call for much higher doses of metals as essential nutrients. Some of the salient issues involved in metals - anaerobic system interactions, including biotransformation, adaptation and engineering aspects of process control are addressed.

ESSENTIALITY AND STIMULATION

One third of all the known enzymes have metals as part of their structure, and this requires that metals be added for activity, (40). When metals are built into the structure of the molecule, they cannot be removed without destroying that structure, e.g. metalloflavoproteins, the cytochromes and ferredoxin (an iron-sulfur protein). In other instances, metals react reversibly with proteins to form metal-protein complexes that constitute the active catalyst. The role of metal is one of stabilizing that catalytically active conformation of the protein (40). Analysis of ten methanogenic (MPB) strains showed the following order of heavy metal composition in the cell Fe>>Zn≥Ni>Co=Mo>Cu>Mn (10) with the iron family considered the most important (Fe, Ni, Co). The importance of iron lies in its redox property and involvement in energy metabolism as cytochrome and ferredoxin. Nickel is essential to hydrogenase, catalyzing $H_2 \leftrightarrow 2H^+ + 2e^-$ conversion, and carbon monoxide dehydrogenase (CODH) which plays an essential role in acetogenic bacteria (4) and in methanogens- Tab. 1. Nickel plays a key role in factor

F_{430} which is present in component C of CH_3-S-CoM methyl reductase system and is probably involved in the terminal step of methanogenesis.
Cobalt is present in corrinoids (4). Cu and Zn are found in superoxide dismutase (SODM); Zn was found in some 70 enzymes (5,6). Recent studies (11) pointed to biological deficiency of Fe, Ni and Co in anaerobic systems in spite of measurable concentrations in sludge.

The importance of heavy metals in acid formers has been established only for some Clostridia. The essentiality of Ni, Se and W can only be inferred indirectly as the presence of these spore formers has been established in anaerobic digestion (44,45).

A generic diagram of methanogenesis versus metal concentration resembles a flattened bell shape curve for an essential metal: stimulatory at low concentration, non-toxic in mid-range and inhibitory at high concentrations . Non-essential metals exert no stimulation and are inhibitory at lower concentrations (13). In municipal digesters metals often exhibit an all or nothing response, i.e. sudden failure when toxic concentration is reached (12).

Table 1. Metals in anaerobic processes

Atom No.	Metal	Function	Reference
Mn		stabilizes methyltransferase (MPB); redox reactions	2
Fe		Cytochrome; ferrodoxin; hydrogenase (H_2ase); CODH; formate dehydrogenase (FDH)	
Co		corrinoids; CODH	3
Ni		CODH; H_2ase in MPB and sulfate reducing bacteria, (SRB); coenzyme A and M; factor F430; growth of MPB	4
Cu		superoxide dismutase (SODM)-MPB	5
Zn		H_2ase; FDH, SODM, nucleic acids metabolism	6
Se		H_2ase; FDH in MPB, Clostridia	7
Mo		FDH, inhibitor of sulfate reducers (SRB)	8
W		FDH; in 20 MPB and in Clostridia	9

Examples of broad and narrow stimulation responses to nickel are presented in Fig. 1 (adapted after Ahring and Westermann -14) . *Methanobacterium thermoautotrophicum* (MT) had a peak at 5 mgL^{-1} and 50% inhibition at 130 mgL^{-1} Ni, while a thermophilic, acetate utilizing (TAM) methanogen tested in parallel had a peak stimulus at 1 mgL^{-1} and inhibition at only 10 mgL^{-1}. It follows that stimulation and toxicity thresholds in anaerobic reactors depend on the type and concentration of the metal and the dominant organism affected. In another study (42) a progressively increasing stimulation of a poultry waste digester gas production rate was accomplished with doses of 1, 10 and 100μM, Ni in spite of appreciable trace concentration of this metal in feed.

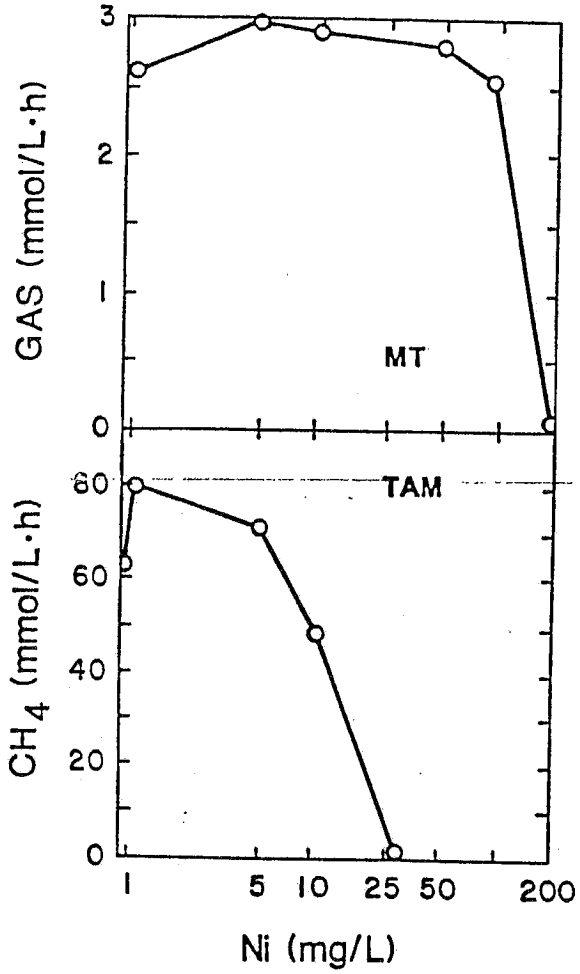

Fig.1

Effects of Ni on two methanogens: MT-gas consumption; TAM-CH_4 generation. (after Ahring & Westermann-ref.14)

Stimulatory effects of Fe^{+2} on sludge digestion have been shown at 10 mgL^{-1} (11) and 28 mgL^{-1} (15) with 1000% increase in acetate utilization rate (AUR). The soluble iron concentration necessary for stimulation was found to vary from 0.5 to 10 mg L^{-1} (11,15). Ten out of thirty tested municipal sludge digestors were found to respond to stimulation by individual or combined doses of Fe, Ni and Co (11). Cobalt at 2 mgL^{-1} stimulated AUR in conjunction with Fe and Ni (11) while only 0.19 mgL^{-1} Co in conjunction with traces of Ni, Mo and Se was sufficient to stimulate grass biomethanation (16).

Iron has been singled out as the most important metal in aggregation of biomass (necessary in sludge bed reactors) due to formation of sulfide precipitates (17) and the ability to tie up cysteine which may promote excretion of extracellular polymers thus promoting granulation

(18). In a study of food industry wastewater three parallel UASB reactors received: R1 - no additives; R2 - calcium and phosphate; R3 - Fe^{3+} 40 mgL^{-1}, 0.5 mgL^{-1} each of Ni and Co (19). Hard, granular sludge developed only in R3 which performed undisturbed through shock COD loads of 5 to 15 kg $m^{-3}d^{-1}$ and velocities of 1.5 to 6 mh^{-1}.

AVAILABILITY OF METALS

Bioavailability of metals is determined by the total metal concentration and environmental conditions in digester such as pH, alkalinity, presence of precipitating anions, complexing by organic and inorganic ligands and the kinetics of precipitation and chelation (20,43). The complexity of the system is further enhanced by the dynamics of sorption and desorption on solids,competition and eviction (substitution) reactions whose rates change with the strength of incoming wastes (21, 22).

Primary inorganic anions in acid-base and gas-liquid equilibria in an anaerobic reactor are formed by the sulfide system, the carbonate system, followed by the phosphate system. All of these are very strongly dependent on reactor pH. Measurements usually show higher soluble content of metals indicating organic and inorganic complexation into soluble forms - beyond the solubility equilibrium (20). Formation of soluble complexes competes with precipitation (37).The soluble fraction amounts to less than 0.5-5% of total metals in sludge digestion (12, 22). The insoluble fraction is considered adsorbed by weakly acidic organic groups with various proposed chemisorption / desorption sequences, eg. Pb>Cu>Cd>Fe=Ni>Zn (22) or Cu>Cd>Zn>Ni (21). Some metals are absorbed into cell (eg. Ni) some are subject to ion-exchange reversibility.

Bioavailability has also been shown to decrease due to chelation, with various sequences of protection against toxicity proposed, eg. EDTA>NTA>aspartate>citrate (26). An example of chelation directly decreasing bioavailability was given for Cd, found toxic to *Methanospirillum hungatei* at 15 µM (36). A dose of 100 µM EDTA fully reversed the inhibition, while citrate or acetate could not. Conversely, the experiment showed poor availability of a chelated metal (Cd used in this study is considered nonessential).

In summary, metals are distributed between soluble and insoluble complexes with varying exchange capacity. Research is needed to elucidate such speciation and its effects on bioavailability (23). Complexation may keep the metal soluble, thereby, enhancing its mobility, but not necessarily bioavailability, which is usually greatest for uncomplexed metals (35).

TOXICITY

At high concentrations metals may act as inhibitors leading to the loss or prevention of enzyme functions. Heavy metals exhibit mostly non-specific, noncompetitive inhibition in relation to substrate utilization or competitive inhibition in relation to another metal. The possible mechanisms of inhibition by metals include: substitution of metallic enzyme cofactors (prostethic group); combining with the sulfhydryl group (-SH) in cysteine or other thiol group-containing-enzymes; and, tight binding to acid groups in the side chains of the amino acids (exhibiting acid reaction such as aspartic or glutamic) in the polypeptide chain (24, 29). This alters enzyme configuration and results in its denaturation. Inhibition of coenzyme M, which occurs ubiquitously in methanogens and contains a mercapto group, is explained by such reaction. Changes in ionic gradient inside the cell also result in toxicity especially when the cell buffering capacity is exceeded. An example of substitution is provided by Cd attacking and replacing Zn at various enzymatic sites (25).

The noncompetitive inhibition (with respect to substrate) cannot be reversed by increasing the substrate concentration. This results in decrease of the specific substrate utilization rate-k (and growth rate µm) proportional to the concentration of metal; and in increase of the half-saturation constant, K_m (24).

Discussion of toxicity has to be conducted with the understanding that measured total metal concentration is meaningless as microorganisms are not at equilibrium with the environment. Rates of uptake, not extracellular concentrations, determine the degree of toxicity (13).

DETOXIFICATION

Excessive concentrations of heavy metals activate a variety of intracellular defense mechanisms: 1.biologically mediated chelation or precipitation; 2. methylation; 3. exocytosis; 4. plasmid mediated resistance.

Precipitation occurs at cell surface through the activity of membrane associated sulfate reductases and occurs after diffusion of sulfides out of the cell (13). Numerous orgainisms have been found to precipitate metal at the cell surface e.g. silver as Ag_2S (13). Effectiveness of this mechanism depends on pH of the reactor (26). Synthesis of ligands for extracellular chelation has also been demonstrated (13).

Biomethylation leading to volatilization and/or precipitation of a metal is known for Hg, Pb, Tl, Pd, Pt, Au, Sn, Cr, As and Se. The synthesis of less polar organometallics allows for elimination by diffusion. Complete detoxification by volatilization of organomercurials into ethane and Hg^0 is possible as well as sulfide mediated precipitation (27):

$$2(CH_3)_3Pb^+ + H_2S \rightarrow (CH_3)_4\,Pb + (CH_3)_2\,PbS\downarrow$$

Magnitude of biomethylation in anaerobic digesters has not been established. Abiotically methylated metals showed decreased affinity for sludge solids (28).

Exocytosis refers to expulsion of a metal from the cell after its chemical / physical inactivation / entrapment in the cytoplasm. Various biopolymers can be synthesized for this purpose (13). Biosynthesis of methallothionen and removal of Cd and Cu by this SH group-containing protein has been demonstrated in eucaryotes (13). Ni-and Cu-trapping mutants have been isolated and shown to bioconcentrate toxic metals intracellularly, approximately 200 times over the surrounding concentration (13). Such cellular inactivation and expulsion mechanism, obtained by acclimation / mutation, was discovered for Zn, Fe, Co, Mo and Hg (13).Energy driven (chemiosmotic) efflux pumps can also be developed which act to prevent excessive accumulation.

Plasmid (R-factor) transmitted resistance to metal toxicity has been found for Ni, Co, Hg and other metals (29). Development of resistance by adaptation to one metal may either enhance or decrease the tolerance to other metals. An alternative to acclimation could be construed by engineering the transfer of resistant plasmids to methanogenic and acid producing cells.

DORMANCY, ACCLIMATION AND EFFECTS OF HRT/SRT

Addition of a slug of heavy metals results in decrease and cessation of methanogenic functions. The cessation is sudden for short solids residence time (SRT) reactors and gradual for longer SRT systems. The reactor is (reversibly) dormant and the length of dormancy (or lag) is proportional to the magnitude of the shock. An example of response to different slug doses of copper added to a laboratory reactor, fed acetate at SRT = 50d, is presented in Fig. 2 (after Parkin & Speece - 38). The study shows accute response with the lag for recovery proportional to the magnitude of the slug (40,50,60 mgL^{-1} Cu). During the period of apparent dormancy ,

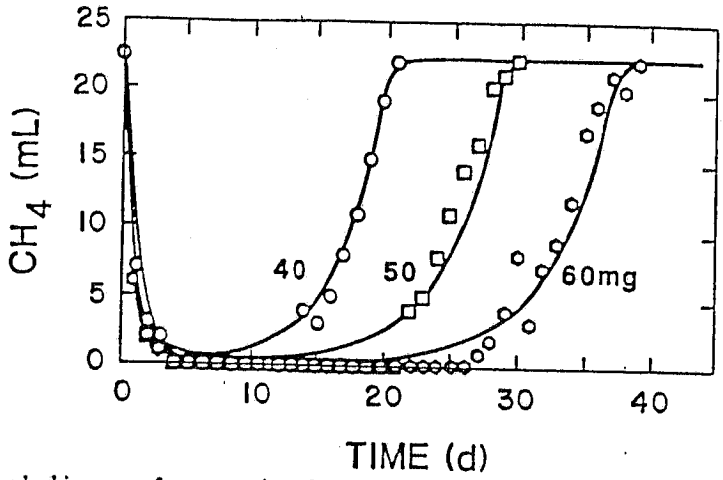

Fig. 2. Measured versus predicted methane production for various copper slugs in mg L^{-1}.

Gradual increase of concentration of a heavy metal may lead to acclimation. Acclimation allows for significant increase in concentration, often an order of magnitude above that found toxic in batch cultures. A generic term - acclimation - may encompass a variety of mechanisms. One is stimulation or turning on and expression of dormant genes. Other may involve: development of intracellular defense mechanisms discussed above; transfer of plasmids; mutation (eg. caused by attachment of some metals to DNA); and a gradual population shift. All are kinetic processes and as such overall acclimation is best conducted in continuous flow reactors, at sublethal chronic concentrations, or depending on HRT/SRT ratio, using short duration shock loads.

Heavy metals affect the growth rate and thus induce the requirement for longer minimum SRT (or HRT). Data from an 8-week long sludge digestion study by Bailey et al. (30) can be presented as in Fig. 3 indicating that up to 300 mgL^{-1} Cr(3+) was tolerated at HRT>21d. After prolonged acclimation another study has shown no adverse effects on sludge digestion at 30d HRT, at 1120 mgL^{-1}Cr (31). Chromium is considered to be one of the least toxic non-essential metals in methanogenesis.

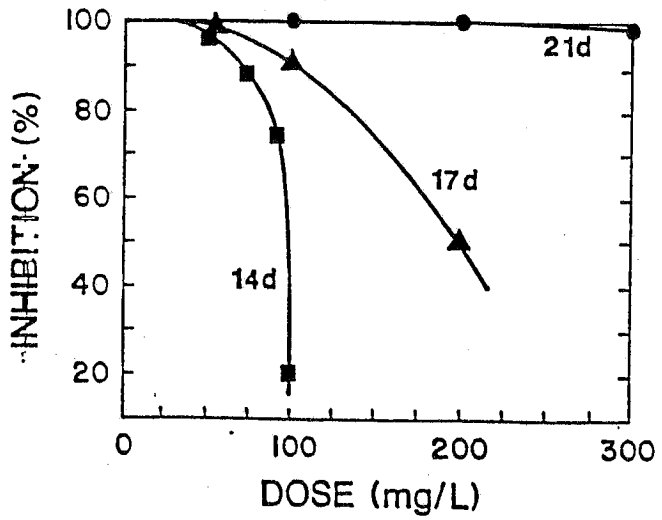

Fig. 3. Effects of HRT on tolerance of Cr(3+)

Fixed film reactors offer long SRT and this is the best safeguard against metal toxicity (32). Slugs are easily washed through and long solids residence time compensates for increased required min-SRT. Series of studies on nickel toxicity (32, 34) have shown: little relation between acute response of unacclimated batch culture and response to both acute and chronic levels of Ni by an acclimated anaerobic biofilter; reversibility of toxicity at doses as high as 2400 mgL^{-1} Ni, provided contact time (HRT) was short (eg. 1 h).

ANTAGONISM AND PROTECTION BY SLUDGE SOLIDS

Antagonism was defined as alleviation of toxic effects of one metal by trace quantity (0.01 M mono- and 0.005 M di-valent metal) of another metal usually through stimulation of methanogenesis rather than direct counter effect (33). Studies (39) showed that retardation of μ_m (with no effect on Y_o, K_m and k_d) by potassium was alleviated by 0.01 - 0.02 M of sodium. The toxicity of Na (decreased μ_m and yield (Y_o) and increased k_d) was reversed by potassium [K]. -as shown in Fig. 4 adapted from Kugelman & McCarty (39)

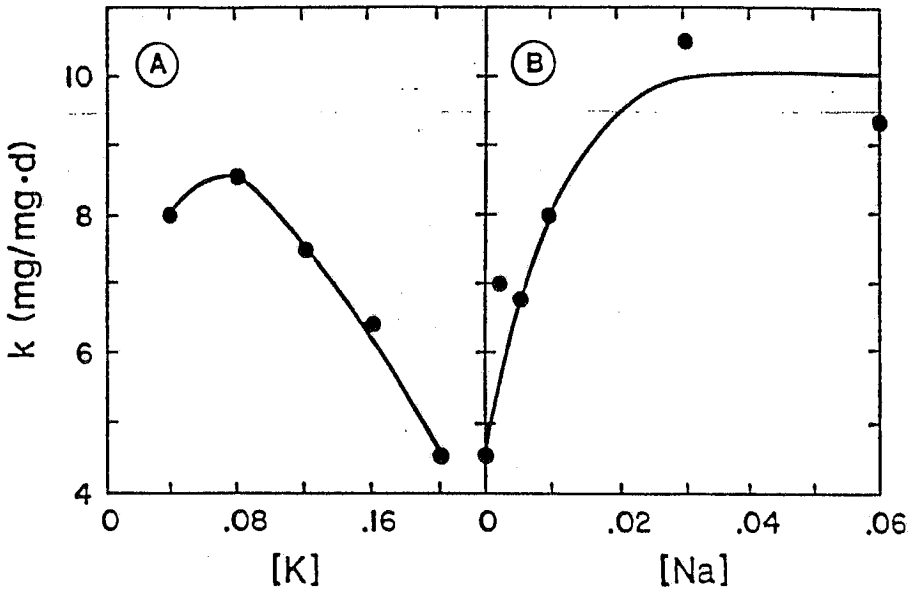

Fig. 4. Effect of sodium on toxicity of potassium: A) Potassium [K] only B) 0.2 M[K] and sodium [Na]

Variety of antagonistic, synergistic and additive effects of combining Ni, Mn, Co, Zn and Hg were described. Any of the three effects could be demonstrated for the same sets of metals depending on concentrations, sequence of addition and type of culture and organic substrate (26,43). In a rare methanogenic study, different antagonistic effects of Ni were shown against Cu and Cd (14) for two methanogens. Fig. 5 illustrates the effects of trace quantities of Ni on toxicity of Cu against MT (adapted from ref. 14). Ni stimulated MT more (Fig. 1) and resulted in stronger antagonistic effect than in the case of TAM (not shown here).

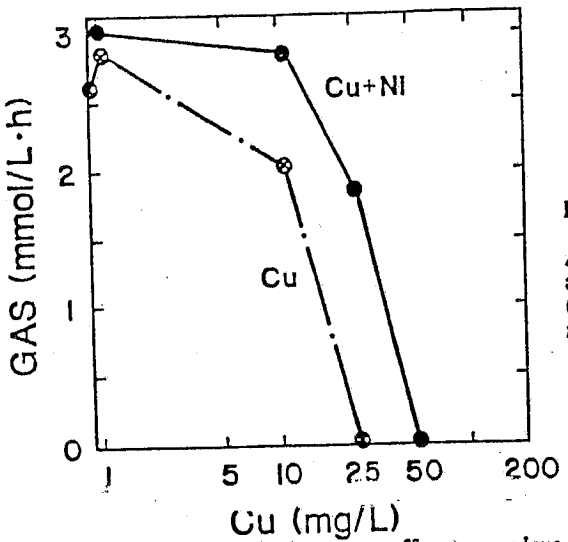

Fig. 5.

Antagonistic effect of trace amounts Ni on Cu toxicity to TM (after Ahring and Westermann - ref. 14).

Presence of sludge solids in the reactor offers tremendous abiotic surface to interact with the metallic ions introduced into the system. Studies of toxicity indicate the need to relate the dose of metals to the amount of solids, i.e. express it in mg M^+ (g dry solids)$^{-1}$ rather than mg L^{-1} (41). Protective effects of sludge solids on methanogenesis was studied by incubating *Msp. hungatei* in HEPES buffer at pH 6.5 (curve A) and in heat treated municipal sludge (B) and (C) from two townships. The study showed 50% inhibition at Cu concentration of 2.3 275 and 65 mgL^{-1}, respectively (Fig. 6 adapted from Jarrell et al., ref. 33). Comparable protection of pure cultures of MPB was offered by sterile sludge against Zn and Ni. The study exposed similar toxicity of Cu and Zn and pointed to 1000% differences in toxicity thresholds reported for the same nickel- methanogen system by different researchers (33, 14) and cited a record resistance of *Mb. formicicum* to 15000 mgL^{-1}Ni in batch culture. These huge differences in thresholds of toxicity observed for just one metal illustrate the complexity of factors responsible for bioavailability.

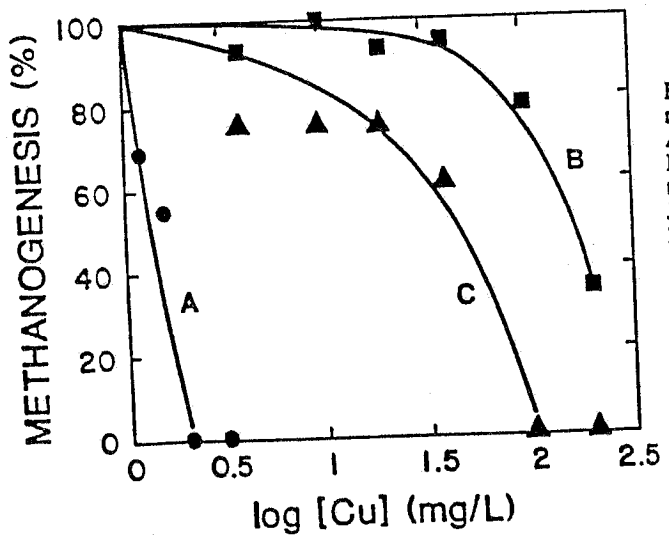

Fig. 6.

Effect of Cu on methanogenesis in: A. HEPES buffer; B. and C. heat treated sludge (after Jarrell et al. - ref. 33).

CONCLUSIONS

Total concentrations of metals in digesters are inadequate indicators of stimulatory or inhibitory thresholds. Variability of metal - reactor biomass interactions, due to complicated detoxifying biochemistry of the anaerobic environment, the effects of acclimation, and abiotic protection offered by sludge solids, necessitate case by case studies of both acidogenesis and methanogenesis.

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