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Toxicity of Heavy Metals to Thermophilic Anaerobic Digestion

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Summary. The effects of heavy metals on the thermophilic digestion of sewage sludge was studied in three semicontinuous digesters step-fed with cadmium, copper and nickel, respectively. The daily gas production, gas composition, the quantitative accumulation of volatile fatty acids, and the distribution of the heavy metals were measured. The fermentations were carried out at 58° C with a retention time of 10 days and an addition of 1.7 g volatile solids/l of reactor volume per day. Nickel was found to be 2–3 times more water soluble than cadmium and copper when the digesters were fed raw sludge containing heavy metals. The three digesters all showed tendencies to acclimate to the heavy metals up to a certain level. 200 mg nickel/l was completely inhibitory while the same response was observed for cadmium and copper at 300 mg/l.

Introduction

Stability of anaerobic digestion requires a balanced activity of the mixed population of bacteria. Process instability is usually indicated by a rapid increase in the concentration of volatile fatty acids with a concurrent decrease in methane production (Kaspar and Wuhrmann 1978; Kroeker et al. 1979; Varel et al. 1977).

Heavy metals are known as elements that may be present in the waste, and they play a significant role in process inhibition and toxicity (Graef and Andrews 1974; Kugelman and Chin 1971; Lawrence and McCarty 1965; Mosey and Hughes 1975).

The toxicity of heavy metals in anaerobic digestion depends upon the various chemical forms which the metals may assume under anaerobic conditions at the temperature and pH value in the digester. For instance, heavy metals in the precipitated form have little toxic effect on the biological system (Hayes and Theis 1978; Lawrence and McCarty 1965; Mosey and Hughes 1975).

Previously, no heavy metal toxicity studies have been carried out on thermophilic digestion. The aim of the present experiments was to study the effect of heavy metals on the activity of the different populations of sludge bacteria during thermophilic anaerobic digestion. During the digestion period the concentration of heavy metals was raised slowly, giving the bacteria a possibility to acclimate. The elements chosen were Cd, Cu, and Ni which have been regarded as environmental hazards, due to their toxicity (Saxena and Howard 1977).

Materials and Methods

Substrate. Two separate batches, each containing 100 l raw sewage sludge, were collected from an activated sludge plant receiving only domestic sewage. The sludge consisted of primary and activated sludge in a concentration of 2.4% total solids and 1.7% volatile solids. After collection the sludge was mixed and frozen in convenient portions and stored at −20° C until required.

Digester Operation. Four 5-l glass bottles, each with a 3-l working volume, were used, one serving as a control. Each digester bottle was fitted with a two-hole rubber stopper, one hole serving as the inlet and outlet for sludge, the other being the outlet for the tubing leading to the gas collection bottle. The digester bottles were placed in a waterbath maintained at 58° C. The gas was collected in 5-l sealed bottles containing a NaCl-solution (30% w/v) acidified to pH 3 with H_2SO_4 to minimize CO_2 solubility. The volume of gas produced was measured by fluid volume displacement into 5-l calibrated bottles.

The development of the thermophilic populations of microorganisms was obtained by incubating a mixture of 1 l of raw sewage sludge and 1 l of mesophilic digested sewage sludge at 37° C in each digester. During the next 10 days the temperature was gradually raised to 58° C. The feeding was then started; 300 ml digested sludge was removed from each digester, replacing it with 300 ml feed sludge heated to 58° C for 1 h in a waterbath. The

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development of similar populations in the digesters was obtained by cross-inoculation of 100 ml digested sludge every day during the first 2 weeks of thermophilic operation.

When the daily gas production was constant solutions of metal salts – $CdCl_2$, $CuCl_2$, and $NiCl_2$ – were added. The chloride salts were chosen to keep as much of the metal as possible dissolved.

For the digesters receiving heavy metals the daily operating routine was as follows: (I) 300 ml raw sewage sludge was mixed with the concentrated heavy metal solution and left to stand for 1 h at 58° C to allow chemical reactions between the salts and the sludge, (II) the overnight gas production was noted after adjustment to standard temperature and pressure, and the gas collecting bottle was refilled from the calibrated bottle, (III) the gas outlet valve was closed and the digester was shaken vigorously followed by an immediate withdrawal of 300 ml digested sludge, using a plastic syringe, (IV) the feed sludge was added, using a syringe, and the digester was shaken again, (V) finally the gas outlet valve was opened. The same routine was carried out for the control digester, except that no heavy metal was added.

Every 10 days the metal concentration was increased to the next heavy metal concentration by pulse-feeding the digesters, and during the following 9 days the digesters were continuously fed metals at this concentration.

Analytical Methods

Volatile solids, total solids, alkalinity, and pH were determined as described in American Public Health Association 1975.

Analysis of Gasses. CH_4 and CO_2 were quantified by use of a Varian Aerograph 1400 equipped with a thermal conductivity detector. The column was made of aluminium and packed with Poropack Q (80/100 mesh). H_2 was used as the carrier gas. The injector, detector, and column were operated at 110° C. The detector current was set at 165 mA. Gas samples of 1.0 ml were collected and injected with a gastight syringe.

Volatile Fatty Acids. Samples to be analysed for volatile fatty acids were acidified to approximately pH 2 with concentrated H_3PO_4 and centrifuged at 4,500 rpm for 30 min. The supernatant was membrane filtered (Millipore HA 0.45 µm poresize) and frozen for later analysis. A microsyringe was used to inject 1 µl of the thawed samples into a Hewlet Packard gas chromatograph (series 5750 G) equipped with a flame ionization detector and a glass column packed with Carbopack. 0.3% Carbowax 20 M/1% H_3PO_4 (Supelco). The injector temperature was 150° C, the detector temperature was 100° C, and the column temperature was programmed as follows: 3½ min at 100° C, temperature rise (10° C/min) up to 175° C, 2 min at 175° C. N_2 was used as the carrier gas.

Heavy Metal Analysis. The distribution of metals in raw and digested sludge was measured at an addition of 0, 10, and 300 mg/l. The soluble fraction was extracted by centrifuging the sludge at 4,500 rpm for 20 min and then filtrating the supernatant. Cheng et al. 1975 have shown that results achieved through this technique do not differ significantly from results attained by membrane filtration. Wet ashing for total concentration determination was carried out by boiling 1 g of dried sludge in 20 ml concentrated HNO_3 until slight dryness. 25 ml 1 N HNO_3 was added, and the sludge was reboiled for 10 min, then cooled and filtrated. The concentrations of Cu, Cd, and Ni were measured on a Perkin Elmer 403 atomic absorption spectrophotometer.

Results

Effects of Heavy Metal Addition on the Digesters

The results of the measured parameters of the four digesters during the experiment are shown in Fig. 1. Before addition of heavy metals the performance of the digesters was constant. This constancy was kept throughout the experiments by the control digester.

Up to 100 mg Cu, Cd, and Ni/l few changes took place. 100 mg of Ni and Cu/l caused an increase in the concentration of volatile fatty acids from 95 and 165 to 130 and 450 mg/l, respectively. The gas production/g of volatile solids added decreased slightly during the first 4–6 days but then again increased to its former level. The addition of Cd obviously did not influence this digester.

At 200 mg/l the Cd digester was still relatively unaffected except for a small increase in the concentration of volatile acids from 102 to 225 mg/l. The Cu digester again showed an acclimational oscillation followed by an increase of volatile acids to 600 mg/l and a decrease of pH to 6.7 and alkalinity to 1,700 mg/l as $CaCO_3$ in the digested sludge. No acclimation was registered in the Ni digester. The gas production decreased to less than 10% of the earlier value. Simultaneously the volatile acids built up to 1,000 mg/l followed by a decrease in pH to 6.5 and alkalinity to 1,500 mg/l as $CaCO_3$. The composition of the gas changed from 66% CH_4 and 29% CO_2 to 48% CH_4 and 44% CO_2.

At 300 mg/l Cu and Cd the digesters were severely toxified, but after few days a certain acclimation could be seen. The contents of volatile fatty acids increased distinctly to 1,300 mg/l in the Cd digester and 1,550 mg/l in the Cu digester while the pH decreased to 6.6 and 6.4, respectively. The alkalinity decreased to 1,500 mg/l in the Cu digester and to 1,900 mg/l in the Cd digester. The CH_4 contents decreased followed by a corresponding increase in CO_2 contents. In the Ni digester the pH decreased to 6.3 and eventually 6.0, which implied that the methanogenic bacteria were unable to carry out further metabolism. This was reflected in the gas composition in which CH_4 accounted for only 20% of the gas produced.

At 400 mg/l all the three digesters were irreversibly inhibited and the volatile acid concentration did not increase further. After 10 days the three digesters had stopped.

Effects of Heavy Metals on the Concentration of Volatile Fatty Acids

During increasing heavy metal addition to the three digesters, the volatile fatty acids accumulated as

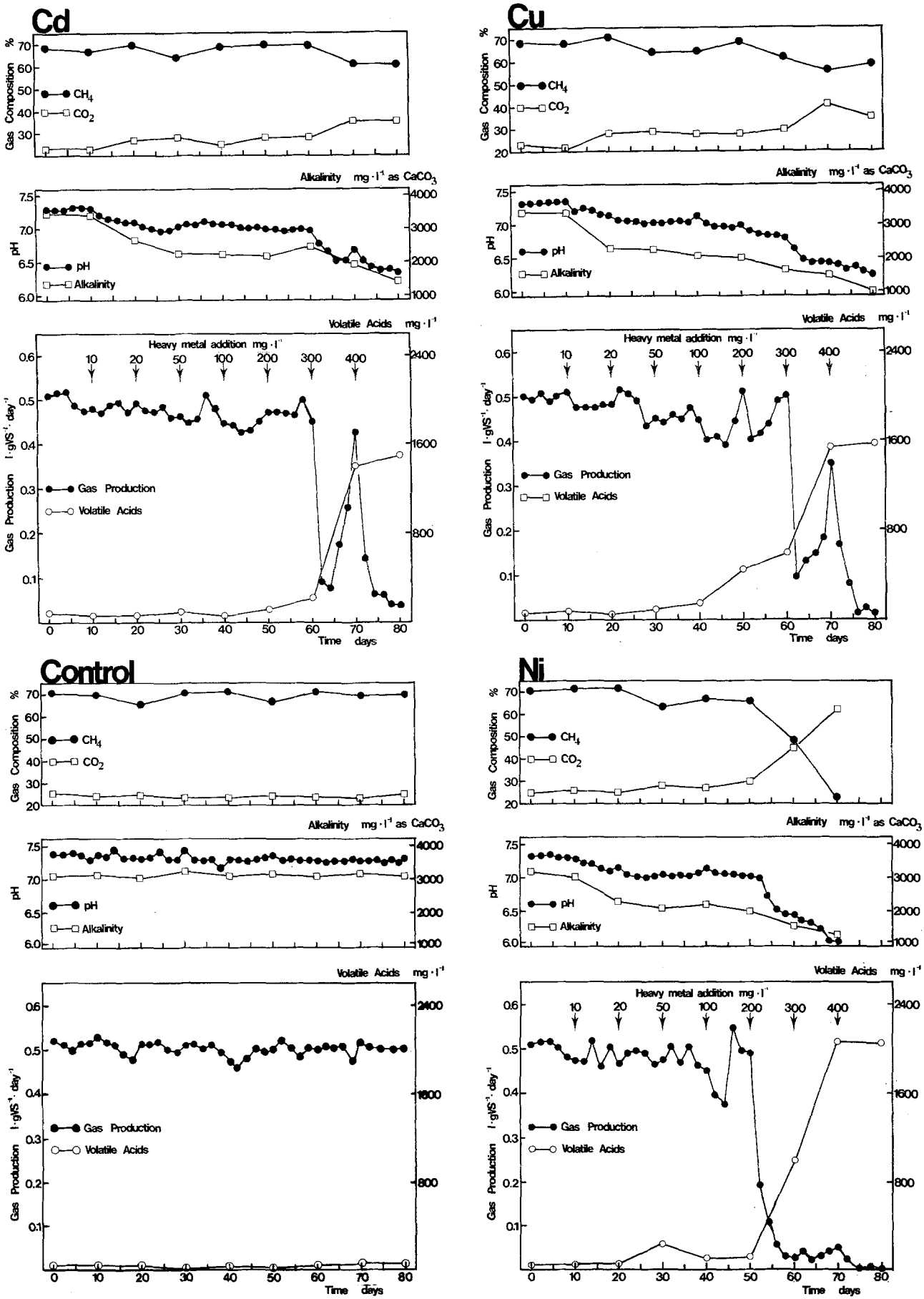

Fig. 1. Effect of increasing Cd, Cu, and Ni concentrations on gas production, gas composition, pH, alkalinity, and total concentration of volatile acids. Gas composition, alkalinity and concentration of volatile acids were measured at each increase in heavy metal concentration. Gas production and pH were measured daily and the values are means of 2 days

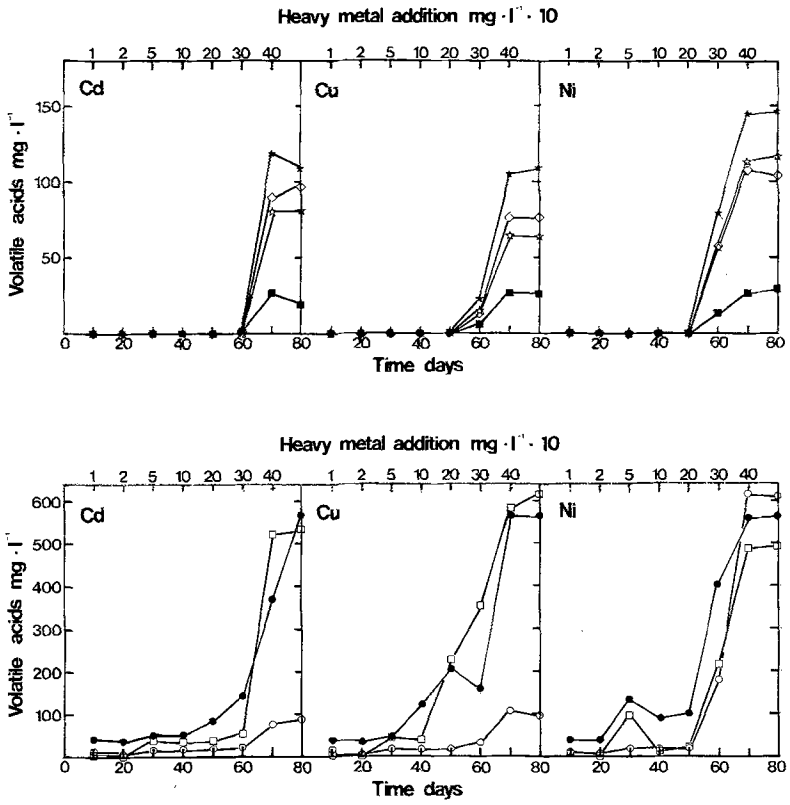

Fig. 2. Effect of heavy metal addition on volatile acid concentration in the three digesters. Symbols: (●) acetate, (□) propionate, (○) butyrate, (★) isobutyrate, (◇) valeriate, (☆) isovaleriate, (■) isocapronate

Table 1. Heavy metal distribution in raw and digested sludge (mg/l)

Heavy metal	Raw sludge			Raw sludge + 10 mg/l			Raw sludge + 300 mg/l			Digested sludge + 10 mg/l			Digested sludge + 300 mg/l		
	Total	Water soluble	%	Total	Water soluble	%	Total	Water soluble	%	Total	Water soluble	%	Total	Water soluble	%
Cadmium	0.3	0.001[a]	0.33	9.82	1.8	18.3	286.44	68.5	23.9	9.06	0.01[b]	0.11	289.21	4.6	1.59
Copper	7.4	0.1	1.35	14.5	1.9	13.1	303.80	59.9	19.7	14.3	0.31	2.17	307.20	8.3	2.70
Nickel	1.0	0.02[b]	2.00	10.7	4.6	43.0	289.20	157.4	54.4	8.40	0.62	7.38	271.30	20.2	7.45

[a] Below the detection limit, i.e., the measured value is less than twice the background noise
[b] Close to the detection limit

shown in Fig. 2. Without any metal added, only acetate, propionate, and butyrate were present. The total contents in all three digesters at this state was 52–55 mg/l. The concentration of volatile acids in the raw sludge was 97 mg/l.

In the Cd digester the volatile acid concentration slowly increased until the addition of 300 mg metal/l which caused a steep increase up to 1,300 mg acids/l. 70% of these volatile acids was constituted of acetate and propionate.

At an addition of 100 mg Cu/l the volatile fatty acids increased significantly. During the following additions all volatile acids accumulated up to a level of 400 mg of Cu/l, where the increase in production rate ceased. At this point 76% of the volatile acids was acetate and propionate.

In the Ni digester the accumulation of acids was initiated at a concentration of 200 mg of heavy metal/l. Unlike the Cd and Cu digesters, butyrate and isobutyrate were produced at a much higher rate. At 300 mg Ni/l 51% of the volatile acids was acetate and propionate, 37% was butyrate and isobutyrate.

Distribution of Heavy Metals

The distribution of heavy metals measured during the experiments is shown in Table 1.

The water soluble fraction of the added metals in raw sludge was clearly reduced after incubation at 58° C for 1 h. At low metal concentrations this was primarily due to the formation of metallo-organic

complexes with the sludge (Gadd and Griffiths 1978). At higher concentrations, beyond this, the metals precipitated as hydroxides and carbonates (Barth et al. 1965; Cheng et al. 1975).

The distribution of heavy metals in the digested sludge indicated that very little was in solution because of sulfide precipitation resulting from bacterial reduction of sulfate and incorporation in the bacterial biomass.

The measurements indicated a distinct difference between the three digesters. In the Ni digester an amount of metal 2–3 times as big as the one in the Cd and Cu digesters was present in a dissolved state. This implies that there was more metal available for participation in the biological processes in this digester. Compared to earlier investigations (DeWalle et al. 1979; Hayes and Theis 1978), executed on mesophilic digestion, our results indicate that, in the thermophilic digestion, the water soluble heavy metal fraction constitutes a larger part of the total metal concentration.

Discussion

A comparison of the results shows that the volatile fatty acids and the alkalinity appeared to be most sensitive to heavy metal toxicity. The decrease in alkalinity reflects the changes in the buffering capacity of the system which is a consequence of the accumulation of volatile fatty acids.

Lawrence and McCarty (1965) found that, when pulse-feeding heavy metals at concentrations of 200 and 400 mg/l to a mesophilic anaerobic sewage digester, gas production decreased much more rapidly than volatile acids increased. In contrast, our studies were executed by step-feeding the digester which enabled the bacteria to acclimate to the increasing metal concentrations.

The methane bacteria are considered a very sensitive group in anaerobic digestion (Chynoweth and Mah 1971; Graef and Andrews 1974; Kroeker et al. 1979; Kugelman and Chin 1971), and failure in the digestion process is mostly accredited to these organisms. Acetate is the precursor of most of the methane produced in mesophilic sludge digestion, and recently Mackie and Bryant (1981) have shown that in thermophilic digestion, acetate is quantitatively more important as a methane precursor. During normal conditions acetate is a major product of the fermentative and acetogenic bacteria. In all three digesters the acetate concentration increased up to a level of 570 mg/l following the increase of the heavy metal concentration. When the acetate concentration in the digesters reached approximately 400 mg/l the gas production was strongly reduced. This corresponds to 200 mg Ni/l and 300 mg Cd and 300 mg Cu/l. The decreased activity of the methanogenic bacteria, therefore, was not caused by substrate limitation, but must be due to heavy metal toxicity.

The degradation of propionate and butyrate to acetate is carried out by the obligate H_2-producing acetogenic bacteria. Infusion experiments with ^{14}C-labelled propionate and butyrate have shown that the two acids accounted for about 23% of the methane produced in a thermophilic digester (Mackie and Bryant 1981). Until now no thermophilic acetogenic bacteria have been isolated but the turn-over of propionate and butyrate indicates that this group of bacteria, as in mesophilic digestion, plays an important role in this type of digestion. The growth of acetogenic bacteria demands a very low H_2 partial pressure to make the acetate production thermodynamically possible (Boone 1982; Bryant 1979; Kaspar and Wuhrmann 1978). The hydrogen consumption by the methanogenic bacteria is the most important process in obtaining this low level, but other groups of bacteria may also contribute to this reduction. The bacteria synthesizing acetate from CO_2 and H_2 appear to have a higher activity in thermophilic digestion than in mesophilic digestion and they are responsible for 3.5–5.3% of the acetate formed (Mackie and Bryant 1981).

In all three digesters the concentration of propionate increased when the heavy metal concentration increased. In the digester receiving sludge enriched with Cu, propionate was the predominant acid after the addition of 100 mg Cu/l, and increased successively during the following additions. In the digester receiving sludge enriched with Cd the accumulation of propionate began at 300 mg Cd/l at which concentration the gas production was reduced significantly. The accumulation of propionate in the Ni digester did not take place until the gas production was totally inhibited and never reached the same level as in the two other digesters. The degradation of propionate is of great importance for the metabolism of organic matter during anaerobic digestion (Kaspar and Wuhrmann 1978). Several other investigations have pointed out that propionate is the first volatile acid accumulating under unfavourable conditions (Boone 1982; Kaspar and Wuhrmann 1978; Varel et al. 1977). The build-up of propionate in our experiments shows that the acetogenic bacteria, which normally degrade this acid, probably are inhibited. This may be due to direct metal inhibition or an accumulation of hydrogen or acetate owing to toxification of the methanogenic bacteria (Chynoweth and Mah 1971). Kaspar and Wuhrmann (1978) found that 4.8 g/l of acetate had to be added to increase the propionate concentration in sludge digestion. Therefore, in our study the build-up of

propionate probably is due to either hydrogen inhibition or direct heavy metal inhibition of the acetogenic bacteria.

The degradation of butyrate is thermodynamically more favourable than the oxidation of propionate and less sensitive to accumulated hydrogen and acetate (Kaspar and Wuhrmann 1978). In contrast to the Cu and Cd digesters, where butyrate concentration was only slightly increased, the butyrate concentration in the Ni digester increased strongly, particularly at the addition of 200 mg Ni/l at which concentration the gas production decreased significantly. This can be caused by difference in heavy metal tolerance of the fermentative bacteria.

Chynoweth and Mah (1971) found that acetate was formed from $^{14}CO_2$ irrespective of methanogenic inhibition. The rise in acetate concentration during our experiments could partly be due to production of acetate by autotrophic acetogenic bacteria. When the methanogenic bacteria are inhibited these bacteria are favoured in the competition for the available hydrogen.

The increase in higher volatile acids caused by the inhibition of the terminal reactions in the digesters shows that the fermentative bacteria are less inhibited than the terminal bacteria by the addition of heavy metals until a level of 400 mg/l, at which concentration the increase in production of volatile acids ceased. In this way, the accumulation of different volatile acids in the digesters reflects difference in tolerance of the fermentative bacteria towards the heavy metals.

In the three digesters the order of decreasing solubility and toxicity is Ni > Cu > Cd on a w/w basis. The difference in availability of the three metals is an important reason of the difference in toxicity during the experiments. Owing to the processes in the anaerobic digester, the available concentration of the specific metal is not necessarily predicted by the water soluble fraction (Buhr and Andrews 1977). Our experiments indicate a certain correlation between water solubility and toxicity of Ni, Cu, and Cd in thermophilic anaerobic digestion.

In all the digesters a tendency of acclimation to the various heavy metals is seen up to a certain concentration. This acclimation may reflect a variety of processes during the acclimation period, such as enzyme induction, development of tolerance, changes in metabolism, etc. (Gadd and Griffiths 1978; Saxena and Howard 1977). McBride and Wolfe (1971) have shown that *Methanobacterium bryantii* is able to methylate arsenate to volatile dimethylarsines. Similar methylations may occur in connection with other heavy metals, e.g., Cu, Cd, and Ni, but this needs further investigations.

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