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SHORT CONTRIBUTION

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Inhibition of methane production from whey by heavy metals – protective effect of sulfide

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Abstract A whey solution was used as a substrate for methane production in an anaerobic fixed-bed reactor. At a hydraulic retention time of 10 days, equivalent to a space loading of 3.3 kg $(m^3\ day)^{-1}$, 90% of the chemical oxygen demand was converted to biogas. Only a little propionate remained in the effluent. Toxicity tests with either copper chloride, zinc chloride or nickel chloride were performed on effluent from the reactor. Fifty per cent inhibition of methanogenesis was observed in the presence of ≥10 mg $CuCl_2\ l^{-1}$ ≥40 mg $ZnCl_2\ l^{-1}$ and ≥60 mg $NiCl_2\ l^{-1}$, respectively. After exposure to Cu^{2+}, Zn^{2+} or Ni^{2+} ions for 12 days, complete recovery of methanogenesis by equimolar sulfide addition was possible upon prolonged incubation. Recovery failed, however, for copper chloride concentrations ≥40 mg l^{-1}. If the sulfide was added simultaneously with the three heavy metal salts, methanogenesis was only slightly retarded and the same amount of methane as in non-inhibited controls was reached either 1 day (40 mg $ZnCl_2\ l^{-1}$) or 2 days later (10 mg $CuCl_2\ l^{-1}$). Up to 60 mg $NiCl_2\ l^{-1}$ had no effect if sulfide was present. Sulfide presumably precipitated the heavy metals as metal sulfides and by this means prevented heavy metal toxicity.

Introduction

In developing countries, huge amounts of industrial wastewater are still discharged untreated to surface waters, contributing significantly to the environmental pollution. Collection and biological treatment of the wastewater is an obligatory requirement. Biogas production from organic pollutants during anaerobic treatment depends on their digestibility (Oi et al. 1982). Due to their presence in municipal and industrial wastewaters, heavy metals contribute significantly to the toxicity in anaerobic systems (Hickey et al. 1989). Low concentrations of heavy metals, however, are essential as trace elements. Their intracellular concentration has to be tightly controlled. Some organisms can acquire heavy metal resistance to a certain extent (Nies 1999). Copper, nickel and zinc have widespread uses in household, scientific and industrial settings, and high concentrations may be toxic for wastewater treatment (Nriagu 1979; Sujarittanonta and Sherrard 1981; Fosmire 1990; Lin 1993; Albek et al. 1997; Ehrlich 1997; Nies 1999). Concentrations of copper, for instance, were 20–120 mg l^{-1} in plating rinse water and 800 mg l^{-1} in wastewater from the copper wire mill process (Patterson 1985). The toxicity of heavy metals depends mainly on the species and its concentration. Other factors such as pH and type of salt may also affect heavy metal toxicity (Albek et al. 1997). The toxicity of heavy metals can be attributed principally to the multiplicity of complexes or clusters they can form (Vallee and Ulner 1972; Butler and Harrod 1989), primarily at the plasma membrane. Plasma-membrane permeabilization by heavy metals has been reported for a variety of microorganisms (Gadd and Mowll 1983). Sulfide has been used to control heavy metal toxicity (Pichon et al. 1988; Jin et al 1998). However, high concentrations of sulfide can inhibit methanogenesis itself or even precipitate essential trace elements (Parkin et al. 1990; Maillacheruvu et al. 1993). In the absence of sulfide, low concentrations of heavy metals were toxic whereas high concentrations could be tolerated if sulfide was provided as a precipitant (Callander and Barford 1983). Knowledge on the effect of heavy metals in industrial wastewater on methanogenesis is still poor (Lin and Chen 1999). The dosages of copper, nickel and zinc for a complete inhibition of

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methanogenesis from industrial wastewater are not clear from the literature. Whether sulfide should be added as a preventive means of heavy metal inhibition to wastewater or whether it could be added as a cure after the inhibiting effect becomes established is also not clear from the literature. Therefore, in this study concentrations of copper chloride, nickel chloride and zinc chloride either causing failure of methane production from whey or even causing failure of acidification were determined. The protective effect of sulfide was also investigated.

Materials and methods

Preparation of the whey solution

Thirty-five grams of whey powder (from Milchwerke Regensburg) was re-dissolved in 1 l tap water to give a chemical oxygen demand (COD) of 33 g l^{-1}. Without further additives, this medium was used as a substrate for methane production in batch and continuous fermentation.

Mixed culture inoculum

An anaerobic glucose-amended mixed culture was used for inoculation of the fermenters. The mixed culture was enriched with anaerobic digester sludge from a beverage company and was maintained at 40 °C in a stoppered conical flask. The mixed culture was fed twice a week with 4 g glucose l^{-1} to maintain methanogenic activity for more than 6 months.

Anaerobic fixed-bed reactor

A glass reactor with a total volume of 4.6 l (height 91 cm, inner diameter 8 cm) was used for batch and continuous experiments. The reactor contained polyethylene/clay sinter-lamellas (0.52 m^2 surface area, Herding, Amberg) as a support material for biofilm formation and was maintained at 37 °C. The reactor was filled with whey solution and was inoculated with 4% of the anaerobic mixed culture. For homogenization the wastewater was re-circulated with a peristaltic pump 3 times h^{-1}. The pH was controlled on-line and adjusted automatically with 1 M NaOH using a membrane pump (ProMinent Dosiertechnik, Heidelberg). The biogas production was measured with a gas flow meter (Ritter, Hanau). After inoculation, a batch fermentation was maintained for 21 days, after which the continuous mode was started. For continuous fermentation, fresh whey solution was pumped into the reactor at the desired flow rate. The medium was stored in a carboy in a refrigerator. The effluent during operation at 10-day hydraulic retention time (HRT) was collected. It was kept at 5 °C and was used as an inoculum for the toxicity experiments.

Anaerobic toxicity assay

Anaerobic toxicity assays were performed with copper chloride, nickel chloride, zinc chloride and sodium sulfide in serum bottles (150 ml total volume) containing 10 ml fresh whey solution. Effluent was collected from the fixed bed reactor (20 ml, HRT = 10 days) and added with 1 ml mixed culture as an inoculum to each bottle to give an initial COD of 14.8 g l^{-1}. Each experiment was done in triplicate with effluent alone, controls (effluent + fresh whey without heavy metal salts or sulfide) and test bottles (effluent + fresh whey with heavy metal salts or sulfide). To study inhibition of methanogenesis or acidogenesis by heavy metals or sulfide, either 10–50 mg $CuCl_2$ l^{-1}, 10–200 mg $ZnCl_2$ l^{-1}, 10–220 mg $NiCl_2$ l^{-1}, or 10–200 mg sodium sulfide l^{-1} was added to the test bottles. To test the protective effect of sulfide in parallel assays, equimolar amounts of sulfide were added either together with the heavy metal salts or 12 days later. Serum bottles, sealed with a rubber stopper and an aluminum cap, were made anaerobic by replacing the air in the headspace with 100% nitrogen at a gas station. The bottles were incubated under gentle shaking at 37 °C. Inhibition was estimated by comparing methane production, residual fatty acids and COD of the test bottles with the control bottles.

Analytical methods

The COD was determined colorimetrically by oxidation of the organic matter in a sulfuric acid/potassium dichromate solution (Wolf and Nordmann 1977). The concentration of Cr^{3+} ions was determined at 615 nm. A calibration curve was prepared with glucose for a concentration range of 0–2000 mg l^{-1}. Methane was determined with a Chrompack gas chromatograph model CP 9001 (Chrompack, Frankfurt), equipped with a thermoconductivity detector (carrier and reference gas flow = 25 ml N_2 min^{-1}). Gases were separated isothermally at 110 °C with a Carboplot 007 column (27.5 m × 0.53 mm). Fatty acids were analysed with a Chrompack model 437 gas chromatograph, equipped with a flame ionization detector (gas supply: H_2, 30 ml min^{-1}, and compressed air, 300 ml min^{-1}). The detector and injector temperature was set at 210 °C. The carrier gas was nitrogen (30 ml min^{-1}) and separation of fatty acids was carried out isothermally at 180 °C on a 2-m Teflon column (1.5 mm inner diameter) packed with Chromosorb C101 (80–100 mesh; Sigma, Munich). Samples were prepared and GC-analyses performed as described by Gallert et al. (1998).

Results

Batch and continuous fermentation of whey to biogas

Whey solution with a COD of 33 g l^{-1} was inoculated with 4% of the mixed culture and was incubated in a batch mode at 37 °C in a fixed-bed reactor. The pH was kept constant at 7 by a titrator unit. COD and fatty acids were determined (Fig. 1). After 4 days, fatty acids began to accumulate up to a maximum of 49 mM for acetate, 179 mM for *n*-butyrate and 46 mM for propionate. Subsequently the fatty acids were slowly degraded to biogas. The methane content of the biogas was 62%. The batch fermentation was continued until the COD

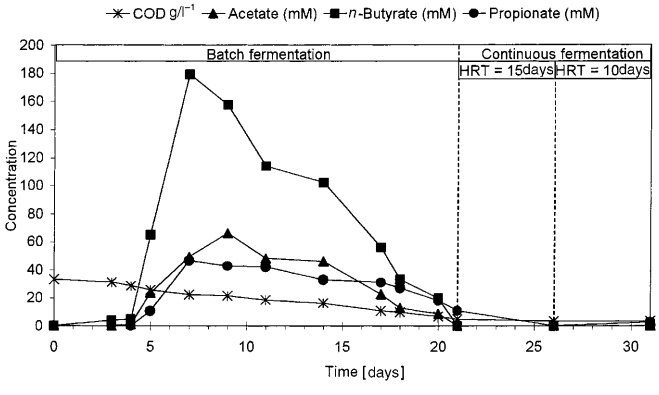

Fig. 1 Batch and continuous fermentation of whey to biogas at a constant pH of 7

decreased to 4.8 g l^{-1}. A complete consumption of acetate and *n*-butyrate (detection limit ≤0.1 mM) was achieved after 21 days, whereas some propionate (4.6 mM) remained. On day 22 continuous fermentation was started with a HRT of 15 days and continued with a HRT of 10 days on day 26. During continuous fermentation at a HRT of 15 days and 10 days the residual COD was 3.4 g l^{-1} (Fig. 1).

Anaerobic toxicity experiments

Table 1 summarizes the residual COD, fatty acid concentrations and methane production in control and test bottles containing whey solution and 10–50 mg $CuCl_2$ l^{-1}, 10–200 mg $ZnCl_2$ $l^{-1}$and 10–220 mg $NiCl_2$ l^{-1}. During 12 days incubation the COD of the controls without heavy metal salts decreased from 14.8 g l^{-1} to 6.7 g l^{-1}. A complete consumption of fatty acids was observed with the exception of small amounts of propionate, which remained undegraded in the medium. Addition of copper chloride at concentrations of 10 mg l^{-1} and 20 mg l^{-1} led to an inhibition of methane formation of 50% and 79%, respectively. A complete inhibition of methanogenesis but not of acidogenesis was caused by 30–40 mg $CuCl_2$ l^{-1}. In the presence of 50 mg $CuCl_2$ l^{-1} even acidogenesis was prevented (Table 1). Analog experiments with zinc chloride or nickel chloride revealed a 50% inhibition of methanogenesis at 40 mg $ZnCl_2$ l^{-1} and 60 mg $NiCl_2$ l^{-1}. Acidification was not inhibited by Zn^{2+} or Ni^{2+}, even at very high concentrations (Table 1).

Table 1 Effect of increasing concentrations of $CuCl_2$, $ZnCl_2$ and $NiCl_2$ on methanogenesis and fatty acid metabolism. Whey solution in tap water was inoculated with the mixed culture described in Materials and Methods and was incubated at 37 °C for 12 days. *COD* Chemical oxygen demand

Heavy metal salt (mg l^{-1})	Methane (ml)	COD (g l^{-1})	Acetate (mM)	*n*-Butyrate (mM)	Propionate (mM)
$CuCl_2$					
Effluent	2.64	3.4	0.0	0.0	6.8
Control	56.4	6.8	0.0	2.0	7.0
10	27.4	9.1	16.4	35.0	20.0
20	11.6	12.1	36.1	36.2	29.2
30	0.0	14.7	18.2	28.0	14.2
40	0.0	14.7	28.0	26.9	20.0
50	0.0	14.8	0.0	0.0	0.0
$ZnCl_2$					
Effluent	2.8	3.1	0.0	0.0	3.0
Control	57.6	6.7	0.0	1.0	5.0
10	51.6	7.1	5.0	6.0	10.0
20	37.7	8.8	9.5	11.7	14.5
40	28.6	10.4	11.6	17.8	16.0
60	11.3	12.1	40.0	38.2	22.0
90	13.6	13.2	46.0	52.5	26.8
120	5.8	13.7	31.8	47.1	38.2
200	3.6	14.4	25.9	29.8	33.6
$NiCl_2$					
Effluent	2.5	3.1	0.0	0.0	2.0
Control	55.7	6.7	0.0	0.0	6.0
10	50.2	6.8	0.0	1.0	12.0
20	44.0	8.4	0.0	2.0	14.6
40	35.5	9.4	8.4	10	14.5
60	27.7	10.4	11.2	18.8	16.8
70	22.8	11.2	18.3	18.8	16.8
100	16.0	12.3	45.1	32.0	18.6
140	8.4	13.1	46.0	51.3	25.6
220	3.6	14.4	24.9	29.8	32.4

Recovery experiments

Effect of sodium sulfide on methanogenesis

Addition of sodium sulfide to the whey cultures at concentrations up to 180 mg l^{-1} revealed no inhibitory effect on methane production, COD degradation or fatty acid consumption (data not shown). Interestingly, addition of 10 mg or 20 mg sulfide l^{-1} led to an increase in methane production by 13.5% and 8.5%, respectively, as compared to controls without sulfide addition. When 220 mg Na_2S l^{-1} were added, there was a slight inhibition of methane production (13.2%). A sodium sulfide concentration of 240 mg l^{-1} caused a reduction of the methane production by about 40% and an accumulation of fatty acids. These experiments showed that sulfide as such was not toxic for methanogenesis at the maximum required concentration for a nearly quantitative precipitation of Cu^{2+}, Zn^{2+} or Ni^{2+} in the experiments shown in Table 1.

Effect of sulfide on restoration of methanogenesis after exposure of the mixed population for 12 days to Cu^{2+}, Zn^{2+}, or Ni^{2+} ions

After 12 days exposure to different concentrations of heavy metal ions, a sodium sulfide solution at concentrations equal to the metal concentration was injected into the serum bottles and incubation proceeded. Except for the assays containing ≥40 mg Cu^{2+} l^{-1}, all assays recovered from the inhibitory effect of the heavy metal salts (Table 2). However the time required for recovery differed markedly and depended on the concentration and type of heavy metal. Whereas in the control assays maximum gas production was reached after 15 days incubation, the bacteria required 38 days or 47 days to restore 100% of their activity in the presence of 10 mg or 20 mg $CuCl_2$ l^{-1}, respectively. Methanogenesis partially recovered with 30 mg $CuCl_2$ l^{-1}, but recovery failed completely at copper chloride concentrations of ≥40 mg l^{-1}. Addition of sodium sulfide to the fermenter samples containing zinc or nickel resulted in a complete restoration of methanogenesis within an extended incubation period. Only at the highest concentrations was methanogenesis not complete (Table 2).

Effect of sulfide addition together with the heavy metals

Triplicate serum bottles containing fresh whey solution were supplemented with either 10–50 mg $CuCl_2$ l^{-1},

Table 2 Effect of sulfide addition on restoration of methanogenesis and fatty acid degradation after exposure of the mixed population to different concentrations of heavy metals. An equimolar concentration of sulfide was added 12 days after incubation of the mixed culture in the presence of heavy metals. Incubation was continued until the same amount of methane was produced in the metal-containing samples as was produced in the metal-free controls after 15 days

Heavy metal salt (mg l^{-1})	Methane (ml)	Recovery (%)	Time (days)	COD (g l^{-1})	Acetate (mM)	*n*-Butyrate (mM)	Propionate (mM)
$CuCl_2$							
Control	76.8	–	15	4.1	0.0	0.0	10.2
10	76.8	100	38	4.5	0.0	0.0	15
20	76.8	100	47	4.6	1.5	0.0	15.0
30	7.9	10.3	52	12.6	49.8	19.4	20.2
40	0.0	failed	–	13.8	55.5	14.6	20.2
50	0.0	failed	–	13.9	19.0	12	13.5
$ZnCl_2$							
Control	74.4	–	15	4.2	0.0	0.0	10.2
10	74.4	100	15	4.2	0.0	0.0	13.6
20	74.0	99.5	17	4.2	0.0	0.0	15
40	73.9	99.4	37	4.4	1.5	0.0	15.0
60	73.8	99.2	35	4.4	1.0	0.0	14.0
80	61.0	82.0	52	5.8	11.0	7.8	20.0
100	47.9	64.4	58	7.8	33.8	22.3	19.3
$NiCl_2$							
Control	72.0	–	15	4.2	0.0	0.0	10.2
10	72.0	100	15	4.2	0.0	0.0	13.6
20	71.6	99.5	16	4.2	0.0	0.0	15
40	71.6	99.5	25	4.3	0.0	0.0	15.0
60	71.5	99.3	33	4.2	1.0	0.0	14.0
80	71.9	99.8	42	4.4	8.0	5.4	19.0
100	61.9	86.0	57	5.8	22.6	18.0	14.6

10–100 mg $ZnCl_2$ l^{-1}, or 10–100 mg $NiCl_2$ l^{-1} and sodium sulfide at an equimolar concentration. Each of the test bottles and the control bottles was inoculated with 1 ml mixed culture and incubated at 37 °C. The time required for a similar methane production as measured in the metal-free controls after 12 days was recorded (Table 3). In the presence of equimolar concentrations of sodium sulfide and copper chloride, copper toxicity could be eliminated up to concentrations of 30 mg l^{-1} and minimized up to concentrations of 50 mg l^{-1}. When up to 100 mg $ZnCl_2$ l^{-1} or 100 mg $NiCl_2$ l^{-1} were present together with an equimolar amount of sodium sulfide, metal inhibition was completely prevented. A slight inhibitory effect only was observed for ≥100 mg $ZnCl_2$ l^{-1} (Table 3).

Discussion

A fixed-bed anaerobic reactor was started with whey as a substrate. After 21 days batch fermentation, the bacteria had converted 85% of the COD to biogas (62%

Table 3 Effect of sulfide addition together with the heavy metals on methanogenesis from whey. Assays with equimolar sulfide and heavy metal additions were incubated at 37 °C until the same amount of methane was produced as had been produced in the metal- and sulfide-free controls after 12 days

Heavy metal salt (mg l^{-1})	Methane (ml)	Recovery (%)	Time (days)	COD (g l^{-1})	Acetate (mM)	*n*-Butyrate (mM)	Propionate (mM)
$CuCl_2$							
Control	54.0	–	12	6.6	3.0	0.0	8.0
10	53.8	99.6	14	6.8	4.0	1.0	11.0
20	54.0	100	17	6.7	4.0	4.0	7.0
30	53.9	99.8	19	6.8	7.0	5.0	7.0
40	47.4	87.8	21	7.8	8.5	15.0	12.2
50	38.9	73	23	8.8	12.8	19.0	19.7
$ZnCl_2$							
Control	57.4	–	12	6.7	0.0	0.0	6.0
10	57.2	99.8	12	6.7	0.0	0.0	5.0
20	57.4	100	12	6.7	0.0	0.0	4.0
40	57.0	99.4	13	6.7	0.0	0.0	8.0
60	57.4	100	18	6.7	0.0	2.0	11.0
80	56.6	98.7	22	6.8	0.0	2.0	11.0
100	51.6	90.0	29	8.1	9.9	6.9	20.0
$NiCl_2$							
Control	58.8	–	12	6.8	0.0	0.0	6.0
10	58.8	100	12	6.8	0.0	0.0	6.0
20	58.6	99.6	12	6.8	0.0	0.0	7.0
40	58.4	99.4	12	6.8	0.0	0.0	8.0
60	58.8	100	12	6.8	0.0	1.0	9.0
80	58.3	99.2	18	6.8	0.0	1.0	10.0
100	58.0	98.8	23	6.9	0.0	3.0	12.0

methane, 38% CO_2, data not shown). During subsequent continuous fermentation at 15 days and 10 days HRT, about 90% of the COD was converted to biogas and a biofilm began to develop on the carrier material. Later, when biofilm formation on the polyethylene/clay sinter-lamellas was completed, about 95% of the COD served for biogas formation (data not shown), as reported by Zellner et al. (1987) in a fixed-bed anaerobic reactor with clay beads. At very low loading, unusually high COD conversions of 99% and 86% were reported for acetate and acetamide, respectively (Guyot et al. 1995). Whereas the methanogenic flora in the whey reactor consumed acetate and *n*-butyrate completely at a space loading of 3.3 kg COD m^{-3} day^{-1}, some propionate remained undegraded and was responsible for the lower efficiency. A similar observation for psychrophilic reactor temperatures was reported by Rebact et al. (1995). The faster disappearance of acetate as compared to *n*-butyrate (if *n*-butyrate was degraded by β-oxidation) may indicate that acetoclastic methanogens were more active than the hydrogenolytic methanogens (Fig. 1), which were mainly responsible for maintaining a low hydrogen partial pressure.

In very low loaded methanogenic reactors, the methanogenic bacteria might have to compete with aerobic bacteria for acetate (Guyot et al. 1994). The oxygen (0.16 mM) was apparently supplied with the feed. A significant contribution of aerobic bacteria for acetate conversion in our whey reactor could be excluded, since oxygen introduction at a much higher loading was negligible.

Industrial wastewater often contains high concentrations of heavy metals. For this reason toxic concentrations of Cu^{2+}, Zn^{2+} and Ni^{2+} ions on methanogenesis from whey were determined (Table 1). Cu^{2+} ions inhibited methanogenesis at much lower concentrations as compared to Zn^{2+} and Ni^{2+} ions. It has been reported that copper decreased the gas production and led to an accumulation of volatile fatty acids in methanogenic reactors (Lawrence and McCarty 1965). With whey as a substrate, methanogenesis failed at a copper chloride concentration of 30 mg l^{-1} and acidification at a copper chloride concentration of 50 mg l^{-1}. Much higher concentrations of zinc chloride and nickel chloride could be tolerated (Table 1). This indicated that methanogenic and acidogenic bacteria were more resistant to zinc and nickel than to copper.

Gas production was considered a useful indicator for monitoring a digester suffering from toxicants (Hickey et al. 1989). Heavy metal concentrations that caused 50% inhibition of methanogenesis during whey methanation indicated that toxicity decreased in the order Cu > Zn > Ni. Similar results were obtained by Lin (1992, 1993) and Lin and Chen (1999). This is, however, not surprising since zinc and nickel are components of several enzymes in anaerobic bacteria (Thauer 1998; Nies 1999). Copper seems to interact easily with radicals in the cell (Nies 1999). Toxicity of heavy metals depends on their concentration. Hayes and Theis (1978) reported that ≥400 mg zinc l^{-1} inhibited anaerobic digestion. Hickey et al. (1989) found that CO_2 production was less inhibited by heavy metals than methanogenesis, indicating a weaker effect of heavy metals on acidogenesis than on methanogenesis. With whey as a substrate, CO_2 was still produced in the serum bottles after methanogenesis had ceased.

Although sulfide is important for precipitation of heavy metals in industrial wastewater, high concentrations of H_2S inhibit methanogenesis (Anderson et al. 1982; Maillacheruvu et al. 1993; Speece 1996). In the whey reactor low concentrations of sodium sulfide (10 mg l^{-1}) slightly enhanced methane production, indicating sulfur limitation (Gerhard et al. 1993). The maximum sodium sulfide concentration tolerated by the whey population without any disturbance was 180 mg l^{-1}. Sodium sulfide could thus be applied to precipitate heavy metals and abolish their toxicity.

An equimolar addition of sodium sulfide 12 days after exposure to copper chloride, nickel chloride or zinc chloride was beneficial for methanogenesis. However a long time was required for a complete recovery, including fatty acid utilization. This confirmed and extended the results of Jin et al. (1998), who reported recovery of an anaerobic system from inhibition by copper through addition of sulfide. The time required for the whey population to recover from the three metals decreased in the order Cu > Zn > Ni. For all concentrations of the metal salts tested, recovery of methanogenesis was much faster and more efficient when sulfide was added simultaneously with exposure to the heavy metals, as compared to the addition of sulfide after exposure to heavy metals. This was in agreement with the results of Jin et al. (1998) for copper. Furthermore our results showed that the bacterial flora completely failed to recover at relatively low copper chloride concentrations of 40 mg l^{-1} and 50 mg l^{-1}, when sulfide was only added after some time of exposure to Cu^{2+} ions, while the addition of sulfide together with the Cu^{2+} ions let the population survive even at a copper chloride concentration of 50 mg l^{-1}.

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References

Albek ML, Yetis Ü, Gökcay CF (1997) Effect of Ni (II) on respirometric oxygen uptake. Appl Microbiol Biotechnol 48: 636–641

Anderson GK, Donnelly T, Mckeown KJ (1982) Identification and control of inhibition in the anaerobic treatment of industrial wastewater. Process Biochem 17: 28–32

Butler IS, Harrod JF (1989) Inorganic chemistry: principles and applications, 1st edn. Benjamin/Cummings, Redwood City, Calif

Callander IJ, Barford JP (1983) Precipitation chelation and the availability of metals as nutrients in anaerobic digestion. I. Application. Biotechnol Bioeng 25: 1959–1970

Ehrlich HL (1997) Microbes and metals. Appl Microbiol Biotechnol 48: 687–692

Fosmire GJ (1990) Zinc toxicity. Am J Clin Nutr 51: 225–227
Gadd GM, Mowll JL (1983) The relationship between cadmium uptake, potassium release and viability in *Saccharomyces cerevisiae*. FEMS Microbiol Lett 16: 45–48
Gallert C, Bauer S, Winter J (1998) Effect of ammonia on the anaerobic degradation of protein by mesophilic and thermophilic biowaste population. Appl Microbiol Biotechnol 50: 495–501
Gerhard E, Butsch BM, Marison IW, Stockar U (1993) Improved growth and methane production conditions for *Methanobacterium thermoautotrophicum*. Appl Microbiol Biotechnol 40: 432–437
Guyot JP, Ramirez F, Ollivier O (1994) Synergistic degradation of acetamide by methanogens and an aerobic Gram positive rod. Appl Microbiol Biotechnol 42: 452–456
Guyot JP, Ferrer H, Florina R (1995) Methane production from acetamide in an upflow anaerobic sludge-blanket reactor based on a synergistic association between an aerobic rod and methanogens. Appl Microbiol Biotechnol 43: 1107–1111
Hayes TD, Theis TL (1978) The distribution of heavy metals in anaerobic digestion. J Water Pollut Control Fed 50: 61–69
Hickey RF, Vanderwielen J, Switzenbaum MS (1989) The effect of heavy metals on methane production and hydrogen and carbon monoxide levels during batch anaerobic sludge digestion. Water Res 23: 207–219
Jin P, Bhattacharya SK, Williama CJ, Zhang H (1998) Effects of sulfide addition on copper inhibition in methanogenic systems. Water Res 32: 977–988
Lawrence AW, McCarty PL (1965) The role of sulfide in preventing heavy meta toxicity on anaerobic treatment. J Water Pollut Control Fed 37: 392–405
Lin CY (1992) Effect of heavy metals on volatile fatty acid degradation in anaerobic digestion. Water Res 26: 177–183
Lin CY (1993) Effect of heavy metals on acidogenesis in anaerobic digestion. Water Res 27: 147–152
Lin CY, Chen CC (1999) Effect of heavy metals on the methanogenic UASB granule. Water Res 33: 409–416
Maillacheruvu KY, Parkin GF, Peng CY, Kuo WC, Oonge ZI, Lebduschka V (1993) Sulfide toxicity in anaerobic systems fed sulfate and various organics. Water Environ Res 65: 100–109
Nies DH (1999) Microbial heavy-metal resistance. Appl Microbiol Biotechnol 51: 730–750
Nriagu JO (1979) Copper in the environment. Part 1. Health effects. Wiley, New York
Oi S, Tamura S, Nakai K, Tanaka T, Taniguchi M (1982) Hydrogen and methane fermentation of rice straw and kitchen leftover. J Ferment Technol 60: 509–515
Parkin GF, Lynch NA, Kuo W, Van Keuren EL, Bhattacharya SK (1990) Interaction between sulfate reducers and methanogens fed acetate and propionate. Res J Water Pollut Control Fed 62: 780–788
Patterson JW (1985) Metals distributions in activate sludge systems. J Water Pollut Control Fed 56: 432–441
Pichon M, Rouger J, Junet E (1988) Anaerobic treatment of sulphur-containing effluents. Water Sci Technol 20: 133–141
Rebact S, Ruskova J, Gerbens S, Lier JB van, Stams AGM, Lettinga G (1995) High-rate anaerobic treatment of wastewater under psychrophilic conditions. J Ferment Bioeng 80: 499–506
Speece RE (1996) Anaerobic biotechnology for industrial wastewaters. Arche Press, Nashville, Tenn
Sujarittanonta S, Sherrard JH (1981) Activated sludge nickel toxicity studies. J Water Pollut Control Fed 53: 1314–1322
Thauer RK (1998) Biochemistry of methanogenesis: a tribute to Marjory Stephenson. SGM special lecture. Microbiology 144: 2377–2406
Vallee BL, Ulner DD (1972) Biochemical effects of mercury, cadmium, and lead. Annu Rev Biochem 41: 91–128
Wolf P, Nordmann W (1977) Eine Feldmethode für die Messung des CSB von Abwasser. Korrespondenzbriefe Abwasser 24: 277–279
Zellner G, Vogel P, Winter J (1987) Anaerobic digestion of whey permeate with suspended and immobilized complex and defined consortia. Appl Microbiol Biotechnol 27: 306–314