



Leaching of iron and toxic heavy metals from anaerobically-digested sewage sludge

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SUMMARY

Heavy metal-loaded sewage sludge was leached abiotically using FeCl_2 and FeCl_3 which are applied in waste water treatment plants to eliminate phosphate and for coagulation. Due to the hydrolyzing nature of ferric iron, ferric chloride (100 mmol L^{-1}) was able to solubilize more than 90% of copper and zinc and more than 80% of cadmium, with an optimal pulp density of 3% (w/v), after 10 h of exposition at 25 °C. Chromium, lead and nickel were solubilized to an extent of 40–70%. With the exception of copper (redoxolysis), all heavy metals monitored were leached following the principle of acidolysis. Chemical leaching with iron resulted in a secondary contamination of sewage sludge (96 g iron per kg dry weight). The insoluble iron compounds which were precipitated or adsorbed to sludge flocks could be resolubilized with oxalic acid (100 mM, $\text{pH} < 3.3$) up to an extent of 90%. Iron was leached by acidolysis and held in solution by complexation with oxalic acid. The pH optimum for the treatment of sewage sludge with 100 mmol L^{-1} oxalic acid was $\sim \text{pH} 3.3$. At this pH an excessive solubilization of nutrient elements and compounds (phosphorus, nitrogen, alkali and alkali earth elements) could be avoided concomitantly leaching 75% iron. Furthermore the hydrophobicity of the sewage sludge was significantly reduced as a result of treatment with iron chloride. *Thiobacillus ferrooxidans* (isolated from arsenopyrite and adapted on sewage sludge) utilized ferrous iron as an energy source in the presence of chloride ions (FeCl_2) as efficiently as ferrous sulphate. No toxic effects of oxalic acid on *T. ferrooxidans* were observed at the prevailing concentration.

INTRODUCTION

To guarantee the supply of detoxified sewage sludge for agricultural use and to realize closed cycle concepts, the operator of a waste water treatment plant should be able to decontaminate metal-loaded sewage sludge. The use of autotrophic microorganisms was reported as an alternative to chemical leaching [3,5,9,11], despite the need to add nutrient substrates like ferrous iron or reduced sulphur compounds for thiobacilli. Most authors favoured the 'classic', acidophilic *Thiobacillus* species (*T. ferrooxidans*, *T. thiooxidans*) because the optimum range of pH for efficient metal leaching was below pH 4 [17,19]. In order to cultivate these species in sewage sludge a preacidification of this substrate is necessary using mineral acid. Recent results show that preacidification with sulphuric acid can be replaced using a mixture of neutrophilic *Thiobacillus* species instead. Especially *T. thioparvus*, *T. novellus* and other species from this genus are able to acidify the untreated sludge suspension because of their high pH optimum for growth [4,6,16]. For this reason the microbial leaching process in co-culture systems will become more attractive.

Ferric iron compounds are used for various purposes such as sludge settling, thickening, dewatering and phosphate precipitation. Because of the low solubility of ferric iron at pH values above 3, high amounts of iron compounds accumulate

in the sludge. Up to 100 g adsorbed or precipitated iron per kg of sludge (dry weight) can be found after chemical phosphate precipitation [8]. Ferric iron which will partly be reduced in the case of anaerobic sewage sludge treatment can be used by *T. ferrooxidans* as energy source by reoxidizing it into the ferric form, which acts itself as a leaching agent, concomitantly acidifying the suspension. The consumption of electrons from Fe^{2+} by *T. ferrooxidans* additionally increases the redox potential, which in turn aids in the solubilization of heavy metals [8].

Some authors assume that iron as a main nutrient salt can meliorize the product sewage sludge [8]. As we do not share this opinion our main interest was to close the iron cycle. One goal of this work was to study the sorption of ferrous and ferric chloride, which are used for chemical phosphate precipitation in waste water treatment. Additionally, chemical leaching of toxic heavy metals by ferrous or ferric iron was investigated because these iron salts (independent from their valence state) act as weak cationic acids. A further important question was by what means and in what quantity iron could be removed from sewage sludge. For this reason oxalic acid which solubilizes iron to a great extent [16] was tested as a leaching agent. *T. ferrooxidans* was studied to utilize ferrous chloride as alternative substrate; this is a product of ferric iron reducing bacteria during anaerobic digestion of sewage sludge. Furthermore, toxic effects of oxalic acid were estimated on the activity of *T. ferrooxidans* in submerged culture.

MATERIALS AND METHODS

Sludge source and metal concentration

Anaerobically-digested sludge (17% dry weight, without lime addition) was obtained from Rossau waste water treatment plant, Innsbruck, Austria. The sludge sample was collected in a polypropylene container. After drying the solid at 80 °C it was pulverized with a pestle (particle size <125 μm) and mixed homogeneously by means of a sample divider. Heavy metal concentrations in sludge as well as levels permitted by the government of the Tyrol for agricultural use are shown in Table 1.

Abiotic experiments

All sorption and leaching experiments were carried out in 500-ml Erlenmeyer flasks containing 80 ml sewage sludge suspension with a defined pulp density of 1, 3 or 5% (w/v). Because of the short incubation time and the thermal pretreatment of the sewage sludge (see above) biotic effects can be neglected for the abiotic experiments. The tests were conducted at a temperature of 25 °C on a gyratory shaker at 200 r.p.m. The incubation time varied between 10 h and 3 days depending on the experiment.

The pulp density of sewage sludge was varied (1, 3 and 5%, w/v) in order to quantify the sorption effects of ferrous

and ferric chloride (each at 100 mmol L⁻¹) in the sludge matrix and to estimate the valence state of the iron in solution.

To estimate the acidolysis and redoxolysis activities of ferrous and ferric chloride (each at 300 mmol L⁻¹) metal solubilization by the iron compounds was compared to metal solubilization by hydrochloric acid (0.9 mol L⁻¹) at a pulp density of 5% (w/v).

Precipitated and adsorbed iron were solubilized with oxalic acid (100 mmol L⁻¹) at pH values of 2, 3, 3.4, 4 and 5 at a pulp density of 3% (w/v).

Analytical methods

The fertilizing value of the sewage sludge resulting from each leaching experiment was estimated. For total analysis of heavy metals, alkali, alkali earth and total phosphorus, sewage was dried (80 °C) and digested with aqua regia according to ÖNORM M 6290 [13]. Solubilized elements were determined by atomic absorption spectrophotometry (Perkin Elmer, Norwalk, CT, USA, standard conditions). Therefore, all samples were filtered (0.2-μm pore-size filter) in order to guarantee a particle-free solution for analysis. Ammonia, nitrate and water-soluble phosphate content as well as the electric conductivity of the sewage sludge suspension were estimated according to ÖNORM S 2023 [13]. The total carbon and nitrogen content was analysed by N/C/S-analyzer (Carlo Erba, Milan, Italy). The pH was measured in all filtrates.

In order to determine the yield of adsorbed or precipitated iron, ferrous and ferric iron in the supernatant fluid was measured spectrophotometrically with 2-2 dipiryridyl [12]. Oxalic acid was quantified by HPLC [16].

TABLE 1

Content of metals and nutrient components of anaerobically-digested sewage sludge (Innsbruck, Austria)

	Metal concentrations (mg kg ⁻¹ dry weight)		
	Rossau	QC 1	QC 2
Cd	6	6	10
Cr	102	300	500
Cu	403	300	500
Fe	11 600	—	—
Ni	36	60	100
Pb	241	300	500
Zn	1034	1200	2000
	Nutrient components (% w/v)		
	Rossau	Min	Max
NO ₃ -N	>0.1		
NH ₄ -N	2.8		
P ₂ O ₅	3.70	3.48	7.62
K ₂ O	0.30	0.48	0.89
Mg	1.23	1.51	5.74
Dry weight	95.90		
Ash content	44.50		
mS cm ⁻¹	2.37	1.69	7.91

QC1, QC2 – permissible concentrations of heavy metals for agricultural use in the Tyrol; Min, Max – minimal and maximal concentrations of nutrient components found in sewage sludge from ten, randomly chosen waste water treatment plants in the Tyrol (Austria, [2]).

Microorganisms

Thiobacillus ferrooxidans (Kfo-6; isolated from an arsenopyrite-containing ore and gradually adapted to sewage sludge) was tested for its chloride sensitivity and its tolerance against oxalic acid. *T. ferrooxidans* was cultivated in 500-ml Erlenmeyer flasks containing 80 ml nutrient solution [22] or sterilized sewage sludge (3% solid dry weight) enriched with ferric chloride (36 mmol L⁻¹) and oxalic acid (100 mmol L⁻¹), respectively. All culture suspensions (pH 3.0; adjusted with sulphuric acid) received a 7% (v/v) inoculum. *T. ferrooxidans* was incubated at 30 °C on a gyratory shaker at 200 r.p.m. for 13 days. To establish chemical reactions (like iron oxidation or oxalic acid precipitation) caused by the supplemented compounds, sterile controls were run with each experiment. Each control was mixed with formaldehyde (5%, v/v). Chemical and biotic tests were carried out in quadruplicate.

RESULTS

Iron as leaching agent

Ferrous chloride (pH 1.5) as well as ferric chloride (each at pH 1.4, 300 mmol L⁻¹) solubilized heavy metals from sewage sludge at least as efficiently as hydrochloric acid (pH 0.8, Fig. 1). Ferric chloride quickly decreased the pH of the suspension to 1.4 (pulp density 5%, w/v) and removed 43% of the chromium, 85% of the copper, 63% of the lead and 60% of the zinc within 2 days. Besides the acidolysis reactions (a high

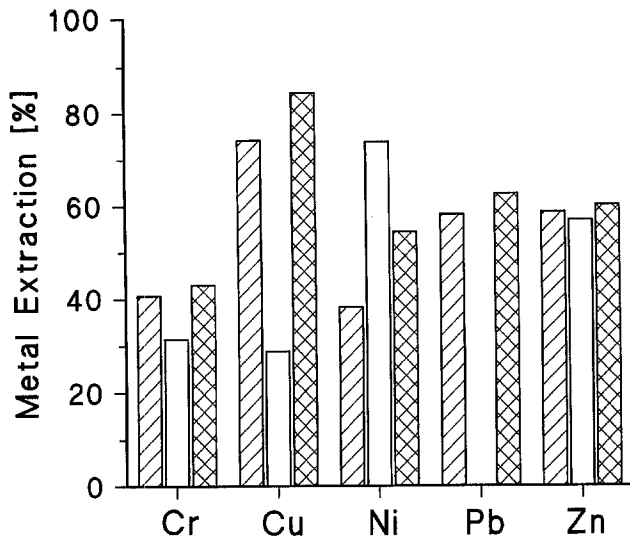


Fig. 1. Mean percentage values of toxic heavy metal extraction from anaerobically-digested sewage sludge (5% pulp density) after treatment with hydrochloric acid (▨; 300 mmol L⁻¹), ferrous chloride (□) and ferric chloride (▩; 100 mmol L⁻¹, each) after 2 days of incubation at 25 °C and 200 r.p.m.

amount of protons is available from the acids) redoxolysis reactions took place in the presence of soluble ferric iron. In comparison to equimolar ferrous chloride, copper solubilization with ferric chloride was more than 50% higher. This result may be due to oxidation of the monovalent cation and the sulphide moiety, respectively.

In order to clarify the solubility and valence status of the iron compounds in sludge suspensions different amounts of sewage sludge (1, 3 and 5%, w/v) were added to solutions containing 100 mmol L⁻¹ ferrous or ferric chloride. With the increase of sludge concentration ferrous and ferric iron were adsorbed or precipitated almost to the same amount (Fig. 2(a)). Extrapolating the linear relationship between pulp density and sorption capacity, iron (100 mmol L⁻¹) should be completely adsorbed at a pulp density of 6.6% (w/v). The effect of sewage sludge on the valence state of iron is also shown in Fig. 2(a). Only small amounts of ferric iron were reduced after 3 days of incubation (6% ferrous iron was found at a pulp density of 1%, w/v). In contrast to ferric iron, ferrous iron did not change its valence state in any of the three sludge suspensions. The low pH values of both iron chloride solutions confirm hydrolysis reaction behaviour of the iron salts. Ferric chloride hydrolyses only two molecules of water instead of three as ferrous iron does, resulting in pH differences in the two iron sludge suspensions [14,15]. Ferric chloride solubilized more than 80% of copper and zinc almost to a sewage sludge concentration of 5% (w/v), whereas ferrous chloride solubilized more than 70% zinc but no copper was found in solution with the same pulp density (data not shown).

In contrast to the results with 300 mmol L⁻¹ ferrous chloride (pH 1.5), with 100 mmol L⁻¹ ferrous chloride solution no acidolysis of copper was observed (pH 4.2, pulp density 5%, w/v).

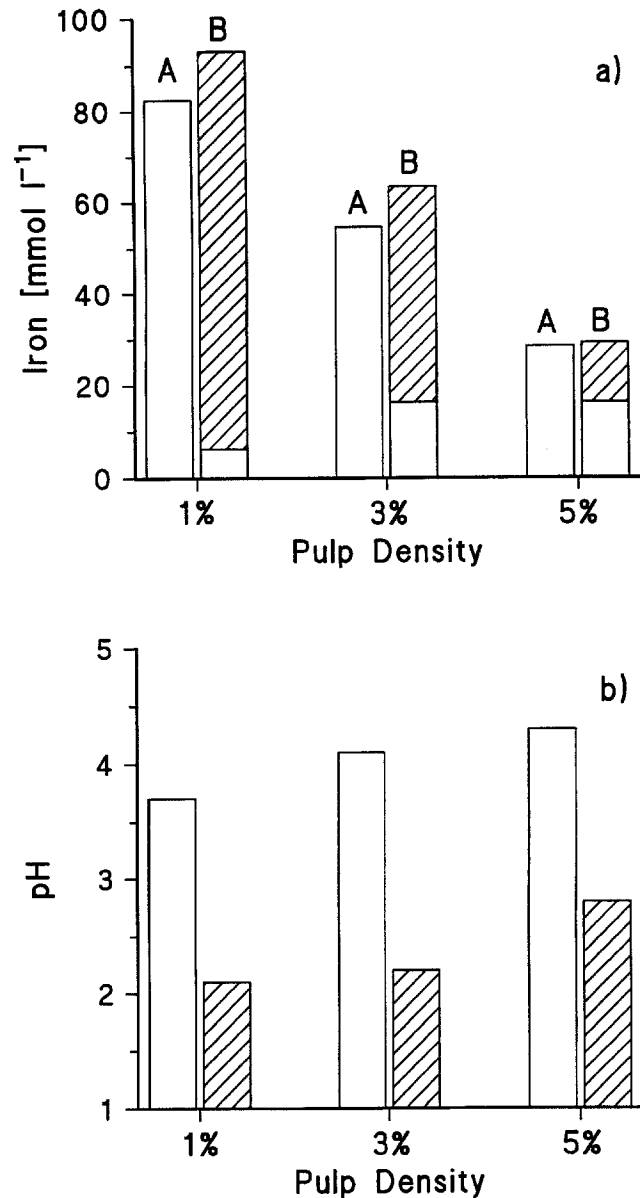


Fig. 2. Estimation of the concentration of ferrous (□) iron and ferric (▩) iron (a) and pH value (b) in the supernatant after leaching anaerobically-digested sewage sludge with ferrous chloride (A) and ferric chloride (B); 100 mmol L⁻¹, each at three different pulp densities (1, 3 and 5% w/v; 25 °C; 200 r.p.m.; 3 days of incubation).

Iron solubilization by oxalic acid

Chemical leaching with ferric iron chloride (100 mmol L⁻¹) resulted in a secondary contamination of sewage sludge with insoluble iron oxides, hydroxides and hydroxosulphates. An eight-fold increase in iron content of the sewage sludge was observed (Table 2). The precipitated or adsorbed iron compounds were best resolubilized with oxalic acid (10 mmol L⁻¹). Up to 80% of the iron contamination was resolubilized by oxalic acid at pH values around 3.0. Whereas ferrous iron did not change its valence during resolubilization by oxalic acid, less than 10% of ferric iron was reduced to ferrous iron.

Above pH 3.3 less than 70% of the iron dissolved and the

TABLE 2

Characterization of anaerobically-digested sewage sludge (3% w/v): untreated, before and after leaching with ferric chloride and oxalic acid (100 mmol⁻¹, each) after an incubation period of 10 h at 25 °C and 200 r.p.m.

Substrate	mg kg ⁻¹ dry weight							g kg ⁻¹ dry weight							μS cm ⁻¹	
	Cd	Cr	Cu	Fe	Ni	Pb	Zn	Na	K	Ca	Mg	C	N	P _t		P _w
Untreated sludge	6	102	403	11600	36	241	1034	3.5	2.3	32.8	18.7	25.5	3.2	14.4	0.7	2370
Substrate leached with 100 mmol L ⁻¹ FeCl ₃	1.0	64	77	96000	23	90	133	0.2	1.4	0.1	7.3	27.9	3.1	11.7	0.0	730
Oxalate (pH 2)	1.1	73	18	9100	20	59	68	0.1	1.3	0.2	7.8	37.9	4.8	1.6	0.0	n.d.
Oxalate (pH 3)	1.2	90	46	10500	23	117	71	0.3	1.6	0.6	7.0	n.d.	n.d.	1.7	0.3	621
Oxalate (pH 4)	1.3	109	95	38000	28	181	148	12.5	1.7	0.1	10.2	30.9	3.2	7.8	0.4	845

Oxalate pH 2, 3, 4 – leaching of the iron chloride treated substrate with oxalic acid at the adjusted pH values; P_t – total phosphorus, P_w – water-soluble phosphorus; n.d. – not determined.

percentage decreased linearly as the pH of the sludge suspension increased. With the exception of pH 5.0 all lower pH values tested solubilized more than 70% iron after 8 h.

Sewage sludge as fertilizer

The acidified sewage sludge, caused by utilizing ferric chloride and oxalic acid as leaching agents, solubilized not only toxic heavy metals but also nutrient ions like alkali and alkali earth and fertilizing components like carbon, nitrogen and phosphorus. Table 2 shows the content of the fertilizing components before and after treatment with ferric chloride and oxalic acid at three different pH values. Besides the water-soluble fraction of alkali and alkali earth salts, the contents of the nutrient elements like calcium, magnesium and potassium were further diminished (up to 50%) in comparison to untreated sewage sludge. Furthermore, the soluble forms of phosphorus were totally reduced by leaching with ferric chloride. With the addition of oxalic acid (100 mmol L⁻¹) more than 75% of the bound and precipitated phosphorus in sewage sludge, respectively, was solubilized at a pH of about 2. Despite the loss of total phosphorus after leaching with oxalic acid (approximately 50% at pH 4.0) the amount of water-soluble phosphorus increased from 0.01 g to 0.4 g P per kg sewage sludge when the pH of the oxalic acid suspension was adjusted to pH 4.0. Total carbon and nitrogen were not significantly changed by these processes. For this reason the fertilizing value of the treated sewage sludge might not be impaired.

Because of the different salt sensitivity of plants the electric conductivity of the untreated and treated sewage sludge suspensions was estimated as an indicator for salinity. In comparison to unleached sewage sludge (2370 μS cm⁻¹) the salinity of sewage sludge treated with ferric chloride was decreased more than 60% (730 μS cm⁻¹). In the three oxalate suspensions (pH 3, 3.4 and 4; adjusted with 5 mol L⁻¹ sodium hydroxide) a small increase of electric conductivity was observed (pH 4, 845 μS cm⁻¹) because of the accumulation of sodium.

Growth of Thiobacillus ferrooxidans in sewage sludge suspension

Figure 3 shows that *T. ferrooxidans* was able to utilize ferrous iron as an energy source in the presence of chloride (70 mmol L⁻¹). The rate of iron oxidation with FeCl₂ was as high as the rate with FeSO₄. Ferrous iron was almost completely oxidized in nutrient solution as well as in sludge suspension after 10 days of incubation. In case of the sewage sludge suspension the concentration of NH₄, the sole nitrogen source, increased slightly during the incubation period. The final pH decrease was almost the same with FeCl₂ or FeSO₄.

To test the effect of oxalic acid on growth of *T. ferrooxidans*, 100 mmol L⁻¹ was added to the nutrient solution and to the sewage sludge suspension, respectively. In the case of the nutrient solution ferrous iron was almost completely precipitated and no growth was observed. The pH value remained

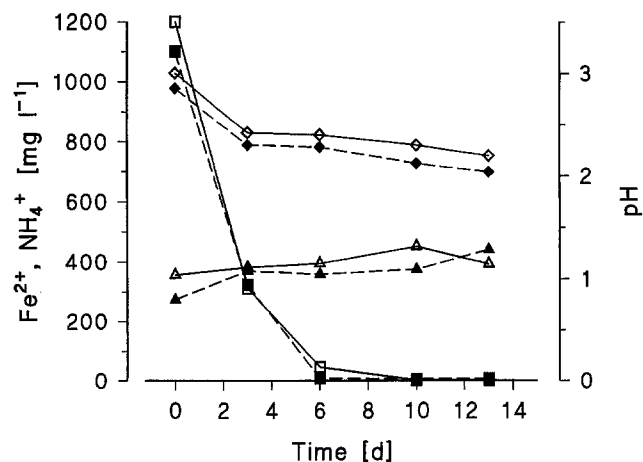


Fig. 3. Time course of ammonia (▲---▲, △---△), ferrous iron concentration (■---■, □---□) and the pH value (◆---◆, ◇---◇) during the leaching of anaerobically-digested sewage sludge (without the addition of oxalic acid) with *T. ferrooxidans* (30 °C; 200 r.p.m.; 13 days of incubation). FeCl₂ (---) or FeSO₄ (—) as energy source (36 mmol L⁻¹, each).

constant at pH 3.0 for the first days of incubation and rose beyond pH 8.5 at the last sampling date (data not shown).

In leaching experiments with sewage sludge (with or without oxalic acid treatment) the suspension's pH decreased below 2.0. In experiments with nutrient solution, 36 mmol L⁻¹ (2011 mg L⁻¹) ferrous iron was added (as the chloride or sulphate). The results of the determination of ferrous iron show that in the case of oxalic acid-treated sewage sludge about 30 mg L⁻¹ Fe²⁺ in solution could be measured at the beginning of the incubation (Fig. 4). The iron concentration increased and reached a maximum value of 150 mg L⁻¹ Fe²⁺ after 6 days, after which it decreased again and after 13 days of incubation the ferrous iron concentration was less than 10 mg L⁻¹. The results of the leaching experiments with sewage sludge in the absence of oxalic acid show a nearly complete consumption of ferrous iron and do not indicate any inhibition of growth of iron-oxidizing bacteria (Fig. 3). NH₄ remained almost constant in the presence of sewage sludge (no oxalic acid added before, Fig. 3) and decreased from 300 mg L⁻¹ to 150 mg L⁻¹ in the presence of oxalic acid-treated sewage sludge (Fig. 4).

DISCUSSION

A successful extraction of toxic heavy metals from sewage sludge depends on (i) the quality and quantity of the metal(s) to be leached; (ii) the proton concentration of the lixiviant used; (iii) the redox potential of the sludge; (iv) the binding strength of the metal(s) to the inorganic and organic compounds; (v) the quality and quantity of the lixiviant; (vi) the pulp density; and (vii) the mean residence time [1,10,17,21]. Ferric chloride is still in use for sludge settling, thickening, dewatering and phosphate precipitation in many waste water treatment plants. Because ferric chloride acts as a weak cationic acid as well as an oxidizing substrate its effect as a leaching agent was tested.

As shown in Fig. 1 significant metal extraction by iron was

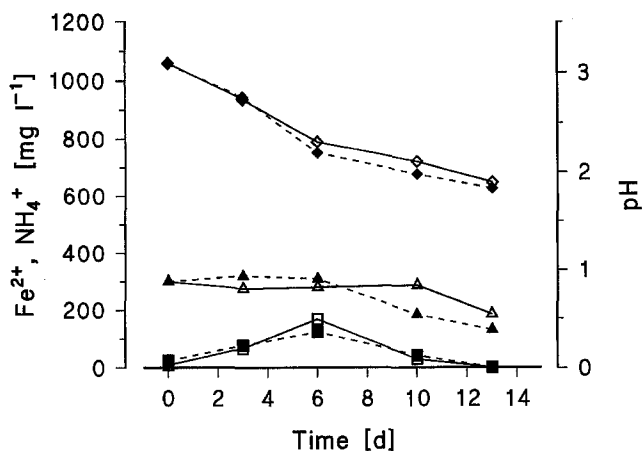


Fig. 4. Time course of ammonia (▲ --- ▲, △ --- △), ferrous iron concentration (■ --- ■, □ --- □) and the pH value (◆ --- ◆, ◇ --- ◇) during the leaching of anaerobically-digested sewage sludge (100 mmol⁻¹ oxalic acid added) with *T. ferrooxidans* (30 °C; 200 r.p.m.; 13 days of incubation). FeCl₂ (---) or FeSO₄ (—) as energy source (36 mmol L⁻¹, each).

achieved for copper and zinc. Because of the different leaching mechanisms for copper and zinc, these elements were taken as indicators of total metal leaching. Zinc was solubilized below pH 3.0 after an incubation period of 3 h [17]. The more acidic the sewage sludge suspension, the more zinc was solubilized. These results suggest that zinc solubilization primarily corresponds to the acidolysis reaction. The leaching behaviour of chromium, nickel and manganese is comparable to zinc, and the leaching efficiency of lead is similar to zinc — lead removal decreased with increased pulp density and pH — but more time was required to approach maximum lead solubilization.

On the other hand the leachability of copper differed from that of the other metals [17,20,21]. Despite acidification of the suspension with hydrochloric acid (pH <1.5) no copper could be observed in solution after an incubation of 2 h at room temperature [17]. With the prolongation of the leaching period copper was solubilized to a lower amount than was zinc.

In the presence of ferric chloride, which acts as an oxidizing agent, the solubility of copper was significantly increased. In comparison to hydrochloric acid leaching, ferric iron solubilized 40% more copper at a pH of 2.0 (data not shown). These results indicate that copper solubilization is caused not only by acidolysis but also by redoxolysis.

The addition of ferric iron chloride (100 mmol L⁻¹) to sewage sludge led to an immobilization of iron, the amount depending on the pulp density of the sewage sludge suspension. In the case of leaching with *T. ferrooxidans*, adding soluble iron as energy source, a further precipitation of iron can be expected. As a consequence the iron content of the sewage sludge may exceed 100 g iron per kg sludge (dry weight basis, [8]). With iron contamination the sludge became distinctly hydrophobic. Spreading such a substrate on arable land seems to be unfavourable in its effects in the long run because of the risk of sealing soil with fine particulate materials. To resolubilize iron from the sludge matrix several organic acids were tested for mobilizing iron precipitates [16]. Oxalic acid proved to be most efficient in resolubilizing iron from sludge bio-flocs. The leaching efficiency exceeded 75% of the total iron content with 100 mmol L⁻¹ oxalic acid (pH <3.5). As a consequence of the oxalic acid treatment the extraction values of heavy metals could be increased (Table 2) and the hydrophobic character of the substrate was significantly reduced. At pH 3.3 high heavy metal extraction was combined with low loss of fertilizing compounds. The total contents of carbon, nitrogen and phosphorus, determining the fertilizing value of the substrate, changed only slightly (Table 2, [18]).

Since the major part of metals in anaerobically-digested sewage sludge occurs in the form of sulphides, leaching with autotrophic ferrous iron oxidizing *T. ferrooxidans* is a promising alternative to chemical leaching. An adaptation to the substrate was a prerequisite to leaching heavy metals from sewage sludge with *T. ferrooxidans* [7]. Ferrous iron was added to the sludge suspension as an energy source for *T. ferrooxidans* because of the low content of metal sulphides in the sludge which only allowed moderate extraction. Irrespective of the salt added, the results of the leaching experiments showed that the species was able to solubilize copper (45%)

and zinc (81%) from sewage sludge in the absence of oxalic acid. Reduction of the ferrous iron concentration and decreasing pH during the incubation period indicated growth of *T. ferrooxidans*. The amount of ammonia as a source of nitrogen remained almost constant. On the one hand *T. ferrooxidans* assimilated NH_4 necessary for growth, and on the other hand the acid-soluble fraction of NH_4 present in the sewage sludge was partly solubilized (pH about 2.0). This may explain the resulting net change of NH_4 concentration (Fig. 3).

In contrast, there was little ferrous iron in solution when applying oxalic acid-treated sewage sludge as a substrate for leaching experiments. This may be due partly to formation of an insoluble Fe^{2+} -oxalic acid complex, precipitation of iron sulphide or sorption to the sludge matrix. The subsequent increase in the concentration of ferrous iron may be due to oxidation of oxalate to carbon dioxide or acidolysis reactions. In view of the time course of the pH and ferrous iron in solution in comparison to uninoculated experiments (data not shown) the leaching activity of *T. ferrooxidans* was not significantly inhibited by oxalic acid in the presence of sewage sludge.

The data presented show the possibility of realizing a closed cycle concept combining abiotic and biotic leaching processes (Table 3). The processes described in this paper may offer the opportunity for waste water operators to provide a high quality sludge suitable for spreading on agricultural land.

TABLE 3

Arguments for chemical and biotic leaching of heavy metals from sewage sludge

Treatment	Advantages	Disadvantages
Iron as additive	Leaching of toxic heavy metals (acidolysis, redoxolysis)	Hydrophobic consistency Secondary contamination with iron
Oxalic acid	Mobilization of iron (acidolysis) Improvement of sludge consistency Mobilization of phosphorus	Additional extraction of nutrient compounds
Leaching active bacteria and fungi	Leaching of toxic heavy metals Nitrogen and phosphorus-storage (biomass) Elimination of pathogenic microorganisms	Not known

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