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Anaerobic Microbial Dissolution of Transition and Heavy Metal Oxides

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Anaerobic microbial dissolution of several crystalline, water-insoluble forms of metal oxides commonly associated with the waste from energy production was investigated. An anaerobic N-fixing *Clostridium* sp. with an acetic, butyric, and lactic acid fermentation pattern, isolated from coal-cleaning waste, solubilized Fe_2O_3 and MnO_2 by direct enzymatic reduction; CdO, CuO, PbO, and ZnO were solubilized by indirect action due to the production of metabolites and the lowering of the pH of the growth medium. Extracellular heat-labile components of the cell-free spent medium obtained from cultures without oxide solubilized a significant amount of Fe_2O_3 (1.7 μmol); however, direct contact with the bacterial cells resulted in the complete dissolution (4.8 μmol) of the oxide. Under identical conditions, the cell-free spent medium solubilized only a small amount of MnO_2 (0.07 μmol), whereas 2.3 μmol of the oxide was solubilized by direct bacterial contact. Reduction of Fe_2O_3 and MnO_2 by *Clostridium* sp. proceeds at different rates and, possibly, by different enzymatic systems. Fe(III) and Mn(IV) oxides appear to be used as sinks for excess electrons generated from glucose fermentation, since there is no apparent increase in growth of the bacterium concomitant with the reduction of the oxides. Dialysis bag experiments with Co_2O_3 indicate that there is a slight dissolution of Co (0.16 μmol) followed by precipitation or biosorption. Although Mn_2O_3, Ni_2O_3, and PbO_2 may undergo reductive dissolution from a higher to a lower oxidation state, dissolution by direct or indirect action was not observed. Also, Cr_2O_3 and NiO were not solubilized by direct or indirect action. Significant amounts of solubilized Cd, Cu, and Pb were immobilized by the bacterial biomass, and the addition of Cu^{2+} inhibited the growth of the bacterium.

Oxides of metals are present in soils, ores, and residues generated from fossil and nuclear fuel cycles. In general, metal oxides are concentrated in the fly-ash and bottom-ash from coal combustion and smelters. They are formed because of the high temperatures and oxidizing conditions used in the process stream. These metal oxides are usually in crystalline forms and are insoluble in water; their fate after disposal in the environment is not known. Organic compounds present in the energy residues and the natural environment can have a significant effect on the solubility and mobility of the metal oxides due to chemical and microbiological action.

Under anaerobic conditions, organic compounds can bring about reductive dissolution of metal oxides from a higher to a lower oxidation state (24). This reduction has a dramatic impact on the solubility and speciation of metals. For example, oxides of Mn(III, IV), Fe(III), Co(III), and Ni(III), when reduced to divalent ions under anoxic conditions, show an increase in solubility by several orders of magnitude (24). Humic substances, catechols, hydroxyquinones, methoxyphenols, resorcinols, ascorbate, pyruvic acid, oxalic acid, amines, anilines, and other naturally occurring organic compounds, including microbial metabolites, have been shown to have redox reactivity (23, 25, 29). Dissolution of cobalt, copper, lead, nickel, and zinc oxides by organic compounds from decomposing plant materials have been reported (13). The role of microorganisms and the mechanisms of microbial dissolution of these oxides have not been clearly established.

Microorganisms also play an important role in the dissolution of metal oxides by direct or indirect action. Direct action involves enzymatic reductive dissolution of the metal oxide, wherein the oxide is used as the terminal electron acceptor, whereas indirect action involves dissolution due to production of metabolites, such as organic acids and chelating agents, and lowering of the pH of the medium. Microbial reduction and dissolution of iron and manganese oxides under anaerobic conditions have been extensively studied (W. C. Ghiorse, Microbial reduction of manganese and iron, *in* A. J. B. Zehnder (ed.), *Biology of Anaerobic Microorganisms*, in press). Most studies deal with the microbial dissolution of amorphous forms of oxyhydroxides of Fe and Mn, and studies with crystalline forms have been very limited (12, 15, 16). Furthermore, we have little information on anaerobic microbial dissolution of heavy metal oxides, particularly those present in the residues from the generation of energy.

An increase in anaerobic microbial activity due to biodegradation of organic compounds present in such residues or contaminated groundwaters can have an appreciable effect on the dissolution of toxic metals from wastes or naturally occurring minerals in subsurface environments. In this paper, we report the mechanism of anaerobic microbial dissolution of several transition and heavy metal oxides.

MATERIALS AND METHODS

Culture conditions. A gram-positive, rod-shaped (2 to 3 by 1.0 μm), sporeforming, nitrate reductase-negative, N_2-fixing *Clostridium* sp. (ATCC 53464) was isolated from coal-cleaning residues. The organism was grown in a medium composed of glucose (5.0 g), glycerol phosphate (0.3 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), $FeSO_4 \cdot 7H_2O$ (2.8 mg), $CaCl_2 \cdot 2H_2O$ (0.5 g), peptone (0.1 g), yeast extract (0.1 g), and distilled water (1,000 ml) (pH 6.8 ± 0.1). The medium was prereduced by boiling and purging with N_2 gas for 15 min to

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remove the dissolved oxygen. It was then cooled under N_2 atmosphere in an anaerobic glove box and dispensed in 40-ml quantities in 60-ml serum bottles. The bottles were closed with butyl rubber stoppers, sealed with aluminum caps, and then autoclaved. The medium was inoculated with 0.2 ml of early-logarithmic-growth-phase cells and was incubated at 24 ± 1°C. The growth of bacteria was measured at 600 nm by using a Bausch and Lomb Spectronic-20 spectrophotometer.

Chemicals. Table 1 lists the sources and purities of the metal oxides. Solid oxides were ground to pass through a 250-mesh sieve and then washed in deionized water several times, dried overnight at 60°C, and stored in a desiccator containing silica gel.

The crystallinity of the oxides was confirmed by X-ray diffraction by using a Philips model XRG3100 analyzer.

Analyses of total gas and organic acids. Total volume of gas, CO_2 and H_2 in the head space of the bottles, and the organic acids in the culture samples were determined as described previously (6).

Mechanism of dissolution of metal oxides. The mechanism of dissolution of the oxides by direct (enzymatic) reduction or indirect (nonenzymatic) action was elucidated by incubating 4.8 μmol of the oxide in 40 ml of (i) uninoculated (control) medium, (ii) inoculated medium, (iii) cell-free spent medium, (iv) autoclaved cell-free spent medium, and (v) synthetic medium. The samples were incubated in 60-ml acid-washed serum bottles fitted with butyl rubber stoppers in N_2 atmosphere for 40 h at 24°C. At the end of the incubation period, the samples were filtered through a 0.22-μm-pore-size Millex filter, and the filtrate was acidified with Ultrex HNO_3 for metal determination. All manipulations, except weighing of the oxides, were performed in the anaerobic glove box.

(i) Dissolution in growth medium. To determine the chemical dissolution of the metal oxides in uninoculated (control) growth medium, prereduced medium was added to acid-washed bottles containing the oxide. The samples were then sealed with butyl rubber stoppers and autoclaved.

(ii) Dissolution in the presence of bacteria. We determined the mechanism of dissolution of the metal oxides by direct contact with bacteria by inoculating the autoclaved medium containing the metal oxide with 0.2 ml of a 24-h-old culture. At the end of the incubation period, total gas production, turbidity, and pH were determined.

(iii) Dissolution by cell-free spent medium. To determine the dissolution of the oxides by extracellular components produced by the bacterium, we prepared a cell-free spent medium. Cells were grown in culture medium in the absence of metal oxide. After 40 h of incubation (optical density at 600 nm, 0.67), the cells were separated from the culture medium by filtration through a 0.22-μm Durapore filter in a 1.5-liter Teflon-coated pressure filtration device (Millipore Corp.) inside the anaerobic glove box. The spent medium was divided into two aliquots. The first aliquot was immediately tested for its ability to solubilize metal oxides.

(iv) Nonenzymatic dissolution. To determine chemical (nonenzymatic) dissolution of metal oxides, the second aliquot of filtered spent medium was autoclaved. The spent medium was added to serum bottles without the oxide inside the anaerobic glove box containing N_2, sealed with butyl rubber stoppers, and autoclaved to inactivate the enzymes. This heat-treated spent medium was filtered again through the pressure filtration device by using a 0.22-μm Durapore filter to remove any denatured cellular material. The pH was then measured. Dissolution of the oxide was tested as described before.

The filtered spent medium and the heat-treated spent medium were checked for cells by direct microscopic examination and for viable cells by incubating an aliquot in a fresh growth medium.

(v) Dissolution by synthetic medium. To determine the effect of acid metabolites and pH of the medium on the dissolution of metal oxides, a synthetic medium was prepared by adding metabolic acids to prereduced growth medium containing only inorganic salts. The metabolic acids, i.e., acetic acid (344 μM), butyric acid (790 μM), and lactic acid (262 μM), were added in the same proportions as found in an inoculated culture medium of *Clostridium* sp. (6). The final pH of the synthetic medium was 3.1.

(vi) Dialysis experiments. Dialysis experiments were performed with those metal oxides which had the potential for reductive dissolution of the metal, such as Co_2O_3, Mn_2O_3, and PbO_2, but dissolution of the metal had not been detected in the filtrate. Metal oxide (4.8 μmol) was placed inside the dialysis bag (Spectrapor [molecular weight cutoff, 12,000]; Thomas Scientific Co. [catalog no. 3787-D10]). The bag was sealed either by placing it around the collar of the butyl stopper to allow direct inoculation or by sealing the bag against one side of the stopper for inoculation into the medium surrounding the bag. The samples were inoculated with 0.2 ml of a 24-h-old culture. At the end of incubation, total gas production was determined. The bottles were opened, and the dialysis bags were transferred to separate serum bottles. The bags and the culture medium were digested separately overnight on a hot plate with 5 ml of concentrated Ultrex HNO_3. Another 5 ml of Ultrex HNO_3 was added, and the digestion was continued until a clear supernatant was obtained. The samples were then analyzed for metals.

TABLE 1. Metal oxides

Metal oxide	Purity (%)	Source
Cadmium oxide (CdO)	99.9	Atlantic Equipment Engineers, N.J.
Cobalt(III) oxide (Co_2O_3)	99.9 (Ultrex)	J. T. Baker Chemical Co., N.J.
Chromium(III) oxide (Cr_2O_3)	98	Aldrich Chemical Co., Wis.
Copper oxide (CuO)	99.9	J. T. Baker Chemical Co., N.J.
Iron(III) oxide (Fe_2O_3)	99.9	J. T. Baker Chemical Co., N.J.
Manganese(III) oxide (Mn_2O_3)	98	Alfa Products, Mass.
Manganese(IV) oxide (MnO_2)	99	J. T. Baker Chemical Co., N.J.
Nickel oxide (NiO)	99.9	Atlantic Equipment Engineers, N.J.
Nickel(III) oxide (Ni_2O_3)	99	Aesar Labs, N.H.
Lead oxide (PbO)	99.9	Atlantic Equipment Engineers, N.J.
Lead(IV) dioxide (PbO_2)	99.9	Alfa Products, Mass.
Zinc oxide (ZnO)	Reagent grade	Aldrich Chemical Co., Wis.

TABLE 2. Effect of metal oxides on the growth and production of organic acids by *Clostridium* sp.

Sample	Growth (OD)[a]	Total gas (ml)	CO_2 (mmol)	H_2 (mmol)	Acid ratio[b]
Control	0.67 ± 0.01	37 ± 3	0.90 ± 0.02	1.05 ± 0	1:1.8:0
CdO	0.69 ± 0.01	29 ± 3	0.97 ± 0.10	1.01 ± 0.11	1:1.6:0
Co_2O_3	0.66 ± 0.01	37 ± 1	ND[c]	ND	1:1.8:0
Cr_2O_3	0.71 ± 0.01	37 ± 3	0.79 ± 0.02	0.80 ± 0	1:1.6:0
CuO	0.66 ± 0.01	32 ± 1	1.07 ± 0.02	1.09 ± 0.02	1:1.8:0
Fe_2O_3	0.68 ± 0.01	33 ± 0	1.07 ± 0.02	1.04 ± 0.01	1:1.8:0
Mn_2O_3	0.67 ± 0.01	32 ± 2	ND	ND	1:1.6:0
MnO_2	0.67 ± 0.01	32 ± 1	1.07 ± 0.02	0.98 ± 0.04	1:1.8:0
Ni_2O_3	0.68 ± 0	35 ± 3	ND	ND	1:1.7:0
NiO	0.69 ± 0.01	36 ± 1	0.90 ± 0.02	1.00 ± 0	1:1.9:0
PbO	0.60 ± 0.01	28 ± 1	1.00 ± 0.02	0.94 ± 0.04	1:2.3:0.8
PbO_2	0.66 ± 0.01	37 ± 1	1.14 ± 0.05	1.18 ± 0.10	1:1.8:0
ZnO	0.73 ± 0.01	32 ± 2	1.07 ± 0.02	1.09 ± 0.04	1:1.7:0

[a] Optical density at 600 nm, ± 1 standard error of the mean.
[b] Acetic:butyric:lactic.
[c] Not determined.

Metal analysis. The soluble metals in the culture medium were determined by atomic absorption spectroscopy (IL 363). Lead was determined by differential pulse polarography using an EG & G polarograph (model 174A and 303SMDE). Reduced forms of iron and manganese were determined as described elsewhere (7, 22).

RESULTS AND DISCUSSION

Growth and organic acid production. In general, the growth of the organism was unaffected by the presence of metal oxide or solubilized metal in the growth medium (Table 2). However, total gas production was less in samples containing CdO, CuO, Fe_2O_3, MnO_2, PbO, and ZnO, in which dissolution of the metal was detected, than in those samples in which there was no detectable dissolution. The ratios of organic acid metabolites produced in all samples were similar, except for the sample containing PbO, which showed significantly increased amounts of lactic acid. The pHs of the uninoculated and inoculated samples at the end of the incubation period were 6.9 and 3.1, respectively.

Cadmium oxide (CdO). Table 3 shows the dissolution of CdO in inoculated, uninoculated, and cell-free spent media. CdO readily dissolved in inoculated (68%), uninoculated (62%), and cell-free spent (72%) media. The amount of soluble Cd detected in solution depended on the presence of cells, cellular components, and the final pH of the medium. Thus, about 62% of Cd was solubilized by the uninoculated medium at pH 6.9, whereas there was complete dissolution (100%) with synthetic medium at pH 3.1. The inoculated medium (final pH, 3.1) and the cell-free spent medium (pH 3.1) had less Cd in solution than the synthetic and the heat-treated cell-free spent media (pH 3.1). These results suggest that dissolution of CdO resulted from changes in the pH of the medium and by the organic acid metabolites. A significant amount of solubilized CdO was attenuated by cell biomass and extracellular components present in the cell-free spent medium. Biosorption of Cd by bacteria has been reported elsewhere (14). The culture medium turned slightly yellow when grown in the presence of CdO. The yellow color was associated with the biomass and was removed when the biomass was filtered through a 0.22-μm-pore-size Millex filter. Cd probably had complexed with cellular

TABLE 3. Dissolution of metal oxides by *Clostridium* sp.

Oxide[a]	% Metal in solution (± 1 SEM) with the following treatment:						
	Medium[b]					Dialysis bag[c]	
	Control	Synthetic	Cell-free spent	Cell-free spent (autoclaved)	Inoculated	Metal and bacteria inside bag	Metal inside and bacteria outside
CdO	62 ± 2	100	72 ± 4	92 ± 1	68 ± 7	ND[d]	ND
Co_2O_3	<1[e]	<1	<1	<1	<1	3 ± 0	<1
Cr_2O_3	<2	<2	<2	<2	<2	<2	<2
CuO	<1	7 ± 3	<1	7 ± 3	<1	<1	5 ± 1
Fe_2O_3	6 ± 1	<1	37 ± 7	9 ± 1	100	ND	ND
MnO_2	<1	<1	2 ± 0	1 ± 0	48 ± 4	ND	ND
Mn_2O_3	3 ± 0	5 ± 1	8 ± 1	4 ± 1	7 ± 1	8 ± 5	3 ± 0
NiO	<2	<2	<2	<2	<2	ND	ND
Ni_2O_3	4 ± 0	3 ± 0	3 ± 1	2 ± 0	3 ± 0	3 ± 0	3 ± 0
PbO	<2	100	74 ± 4	ND	38 ± 4	ND	ND
PbO_2	<2	ND	6 ± 1	8 ± 4	<2	2 ± 0	3 ± 1
ZnO	12 ± 0	98 ± 10	99 ± 1	100	100	ND	ND

[a] Metal (4.8 μmol) was added to 40 ml of medium; soluble metal in the medium was determined after 0.22-μm filtration.
[b] The pH was 3.1 for all treatments except the control medium, which was 6.9.
[c] Metal content was measured in the culture medium outside the dialysis bag.
[d] ND, Not determined.
[e] <, Values are below detection limit.

components and formed a Cd-S bond similar to that of the metallothionines (8). The toxicity of Cd(II) to the bacterium was tested by adding 4.8 μmol of soluble $Cd(NO_3)_2$ to an early-log-phase culture. No effect on growth was noted.

Cobalt(III) oxide (Co_2O_3). Cobalt(III) oxide showed a slight dissolution into the medium only when bacteria and the oxide were present inside the dialysis bag (Table 3). Therefore, direct contact may be required for reductive dissolution of Co_2O_3; additional studies are needed to verify this observation. Biosorption of the metal may also occur, since no cobalt was detected in solution when it was incubated in medium containing Co_2O_3. Organic compounds can reduce Co(III) to Co(II) and thus increase the solubility of the metal (21).

Chromium(III) oxide (Cr_2O_3). Chromium(III) oxide potentially can be reduced to Cr(II). However, it is stable in aqueous solutions only for a few hours, even in the absence of oxygen (3). The experimental data indicate that Cr_2O_3 was quite insoluble in all of the treatments (Table 3).

Copper oxide (CuO). Copper oxide was slightly soluble (about 7%) in synthetic and autoclaved cell-free spent medium (Table 3). Dissolution of CuO in uninoculated, inoculated, and cell-free spent media was below the detection limit (<1%). Although CuO may undergo reductive dissolution from Cu(II) to Cu(I), the cuprous ion is very unstable (3). Experiments with dialysis bags suggest that the small amount of CuO dissolution was due to chemical action brought about by the organic acids and low pH (Table 3). There was a significant biosorption of Cu by cells and the cellular components present in the cell-free spent medium. The addition of 4.8 μmol of soluble $CuCl_2$ to 40 ml of the growing culture immediately inhibited the growth of the organism, and 31% of the added Cu was attenuated by the biomass. Cu not only is toxic to bacteria but also is known to bind to cells (2).

Iron(III) oxide (α-Fe_2O_3). Iron oxide (hematite) was relatively insoluble (6%) in uninoculated medium (Table 3). In the synthetic medium, there was no dissolution (below detection limit, <1%) of the oxide, suggesting that the pH and organic metabolites are not responsible for the dissolution of Fe_2O_3. Dissolution of the oxide in the cell-free spent medium was significant (approximately 37%), whereas in the presence of actively growing cells, it was complete. Only low amounts of the oxide (about 9%) were solubilized by autoclaved cell-free spent medium.

Dissolution of Fe_2O_3, therefore, is due to enzymatic action as well as to the heat-labile extracellular components present in the spent medium. Dissolution of crystalline forms of Fe_2O_3 by such extracellular components from cultures grown in the absence of the oxide has not been reported previously. Furthermore, the results suggest that direct cell contact is not necessary for reductive dissolution of Fe_2O_3, contrary to what has been observed by others (17, 28). This may be due to differences in the experimental methods. Munch and Ottow (17) and Tugel et al. (28) used dialysis bags with an approximate pore size of 2.5 μm, whereas we used a 0.22-μm filter, which has a pore size of about 200 μm. Extracellular polymers, including some proteins, pass through the membrane filters more freely than through the dialysis bags. Similarly, Jones et al. (11) reported that extracellular components present in the cell-free growth medium of sediment bacteria (after 0.2-μm filtration) which were grown in the presence of amorphous $Fe(OH)_3$ solubilized only about 30% of Fe. Amorphous Fe(III) hydroxides are readily reduced by sedimentary bacteria (15) and *Clostridium butyricum* (16). The dissolution of iron oxides decreased with increasing crystallinity [$Fe(OH)_3$ > hematite > geothite] (11, 16).

Manganese(IV) oxide (MnO_2). Little dissolution of MnO_2 (pyrolusite) occurred in the uninoculated medium (below detection limit, <1%), cell-free spent medium (2%), autoclaved cell-free spent medium (1%), and synthetic medium. However, in the presence of actively growing cells (Table 3), 48% was solubilized, indicating that there was direct enzymatic reductive dissolution of Mn(IV) to Mn(II). Bioaccumulation of Mn(II) was not evident. The addition of Mn(II) (4.8 μmol of $MnCl_2$ per 40 ml) to the growing culture had no effect on the growth of the organism.

Reductive dissolution of MnO_2 directly by microbial enzymes, indirectly by microbial metabolites, and by naturally occurring organic compounds has been extensively studied (5, 22, 23). In this study, dissolution of Mn(IV) oxide by *Clostridium* sp. was due to direct enzymatic reduction; the metabolites produced by the organism had no effect on dissolution. A significant amount of MnO_2 was readily reduced and dissolved only in the presence of cells. These results support the findings of others that contact with the bacterial cell is necessary to bring about dissolution.

Manganese(III) oxide (Mn_2O_3). Dissolution of Mn_2O_3 was not as pronounced as that of MnO_2 (Table 3). There was a slight increase in the dissolution of Mn_2O_3 in cell-free spent medium, inoculated medium, and dialysis bag experiments compared with dissolution in the uninoculated (control), synthetic, and autoclaved cell-free spent media. This may be due to the presence of other forms of Mn (i.e., MnO_2) present as impurities with Mn_2O_3. There is no information on the biological dissolution of the crystalline form of Mn(III) oxide, but reductive dissolution of amorphous Mn(III) by naturally occurring organic compounds (23) and bacteria (5) has been reported. Lack of dissolution of Mn_2O_3 may also be due to its refractory nature.

Nickel oxides (NiO and Ni_2O_3). Ni(II) oxide showed no dissolution with any of the treatments (Table 3). However, Kee and Bloomfield (13) reported that freshly prepared amorphous NiO [most probably existing as $Ni(OH)_2$] was solubilized by decomposing plant residues, whereas crystalline NiO was resistant to dissolution. Since the NiO used in this study also was crystalline, our results confirm that this form of oxide is refractory. Soluble Ni from dissolution of Ni_2O_3 was not detected with any of the treatments, even though the reduction of Ni(III) to Ni(II) is a possibility.

Lead oxide (PbO). PbO was insoluble (below detection limit, <2%) in uninoculated (control) medium at pH 6.9, completely soluble (100%) in synthetic medium, and soluble (74%) in cell-free spent medium (Table 3). Dissolution of PbO in an autoclaved spent medium was not determined. The difference between the dissolution of PbO in synthetic medium and in cell-free spent medium is due to adsorption of solubilized lead by the cellular components present in the latter. In the inoculated medium, about 40% of Pb was detected as soluble Pb in the medium. Lead forms weak complexes with organic acids (20), and it has been shown that lead from PbO is complexed by the organic metabolites produced by the bacterium (6). The data show that significant amounts of solubilized Pb were immobilized by the bacterial biomass and the extracellular components of the cell-free spent medium. Biosorption of Pb by microorganisms has been reported previously (1, 6, 27).

Lead dioxide (PbO_2). A small amount of PbO_2 dissolution (Table 3) was observed only in the cell-free spent medium and the autoclaved cell-free spent medium. PbO_2 has the potential for undergoing reductive dissolution. It also is

TABLE 4. Mechanism of dissolution of metal oxides by *Clostridium* sp.

Metal oxide	Oxidation state of metal used	Mechanism of action[a]	
		Expected	Observed
CdO	2^+	Indirect	Indirect
Co_2O_3	3^+	Direct ($Co^{3+} \rightarrow Co^{2+}$)	?
Cr_2O_3	3^+	Indirect	None
CuO	2^+	Indirect	Indirect
Fe_2O_3	3^+	Direct ($Fe^{3+} \rightarrow Fe^{2+}$)	Direct
MnO_2	4^+	Direct ($Mn^{4+} \rightarrow Mn^{2+}$)	Direct
Mn_2O_3	3^+	Direct ($Mn^{3+} \rightarrow Mn^{2+}$)	None
NiO	2^+	Indirect	None
Ni_2O_3	3^+	Direct ($Ni^{3+} \rightarrow Ni^{2+}$)	None
PbO	2^+	Indirect	Indirect
PbO_2	4^+	Direct ($Pb^{4+} \rightarrow Pb^{2+}$)	None
ZnO	2^+	Indirect	Indirect

[a] Indirect, Dissolution due to metabolites or lowering of pH of medium; direct, enzymatic reductive dissolution of metals from higher oxidation state to lower oxidation state; none, no significant dissolution of metals detected.

slightly soluble in acetic acid, one of the metabolites produced by the bacterium. The slight solubility of PbO_2 in acetic acid is due to the weakly basic character of the oxide (19). However, the small amount of soluble lead from PbO_2 was not detected in the presence of cells and probably bound to the cell biomass. Dialysis experiments showed no evidence for dissolution of PbO_2 by direct or indirect action.

Jones et al. (10) found no evidence of reduction of PbO_2 to Pb(II) by a glucose-fermenting organotroph (*Vibrio* sp.) or an H_2-utilizing anaerobic lithotroph. However, dissolution of PbO_2 by decomposing plant residues was reported by Kee and Bloomfield (13).

Zinc oxide (ZnO). Zinc oxide was slightly soluble (12%) in uninoculated medium and was completely soluble in synthetic, cell-free spent, and inoculated media (Table 3). These results show that the dissolution of ZnO is primarily due to the effects of organic acids and low pH. Biosorption of solubilized Zn by cell biomass was negligible.

The results presented herein show that dissolution of insoluble metal oxides by anaerobic microorganisms can occur by direct enzymatic reduction of metals from a higher to a lower oxidation state or by indirect action due to microbial metabolites and lowering of the pH of the medium. Table 4 summarizes the mechanism of dissolution of several metal oxides by *Clostridium* sp. Fe_2O_3, MnO_2, and possibly CO_2O_3 were solubilized by direct action, whereas CdO, CuO, PbO, and ZnO were solubilized by indirect action. Cr_2O_3, Mn_2O_3, NiO, Ni_2O_3, and PbO_2 were not solubilized by direct or indirect action. The enzyme involved in the reduction of Fe oxides and Mn oxides is not known, but it appears that different enzymes, as well as heat-labile extracellular components, are involved. It is interesting to note that Fe_2O_3, but not MnO_2, was solubilized by cell-free spent medium. Additional studies are needed to determine the active principles involved in the dissolution of these oxides. Further, our data suggest that Fe_2O_3 and MnO_2 are used as sinks for excess electrons generated from glucose fermentation, since no increase in growth of the bacterium or total gas production was observed concomitant with the reduction of the oxides.

We can make no generalizations with respect to the dissolution characteristics of the oxides tested because of the complex chemistry involved, which is further complicated by biosorption and precipitation occurring simultaneously in the culture medium. However, for certain oxides, their mineralogy and form (amorphous or crystalline) may partially determine their solubility (21). Amorphous or poorly crystallized oxides of Fe and Mn are more readily reduced by microorganisms than highly crystalline minerals (4, 5, 9, 11, 15, 17). Crystalline Fe(III) oxides are generally more thermodynamically stable than their noncrystalline counterparts; thus, one can assume that the lower the degree of crystallinity, the higher the extent to which the pedogenic iron oxides may be reduced (18, 26). With the exception of CdO, the oxides used in this study are quite insoluble in water and are crystalline in nature.

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