Microbial Formation of Manganese Oxides

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Microbial manganese oxidation was demonstrated at high Mn^{2+} concentrations (5 g/liter) in bacterial cultures in the presence of a microalga. The structure of the oxide produced varied depending on the bacterial strain and mode of culture. A nonaxenic, acid-tolerant microalga, a *Chlamydomonas* sp., was found to mediate formation of manganite (γ - $MnOOH$). Bacteria isolated from associations with crude cultures of this alga grown in aerated bioreactors formed disordered γ - MnO_2 from Mn^{2+} at concentrations of 5 g/liter over 1 month, yielding 3.3 g of a semipure oxide per liter. All algal-bacterial cultures removed Mn^{2+} from solution, but only those with the highest removal rates formed an insoluble oxide. While the alga was an essential component of the reaction, a *Pseudomonas* sp. was found to be primarily responsible for the formation of a manganese precipitate. Medium components—algal biomass and urea—showed optima at 5.7 and 10 g/liters, respectively. The scaled-up culture (50 times) gave a yield of 22.3 g (53 mg/liter/day from a 15-liter culture) of semipure disordered γ - MnO_2 , identified by X-ray diffraction and Fourier transform infrared (FTIR) spectroscopy, and had a manganese oxide O/Mn ratio of 1.92. The Mn(IV) content in the oxide was low (30.5%) compared with that of mined or chemically formed γ - MnO_2 (ca. 50%). The shortfall in the bacterial oxide manganese content was due to biological and inorganic contaminants. FTIR spectroscopy, transmission electron microscopy, and electron diffraction studies have identified manganite as a likely intermediate product in the formation of disordered γ - MnO_2 .

Many disordered manganese oxides have an indeterminate structure (2, 33). The crystalline phases of these oxides are complex because of structural intergrowths, lattice defects, cation vacancies, random octahedral-unit (MnO_6) distribution, and the amorphous nature of the oxide (1, 25). The structurally disordered phases tend to be more electrochemically active than common oxides (e.g., pyrolusite and cryptomelane) and as such are preferred as depolarizers in dry-cell batteries (12). At present, electrolytic or chemically formed disordered manganese oxides are used in batteries, as the natural mined ores have lower activities and are a scarce resource (18, 31).

Although it is unclear why structurally disordered oxides have higher electrochemical activity (26), it has been suggested as being a result of (i) better proton diffusion within the oxide particles, (ii) greater purity of the MnO_2 content, (iii) greater particle size, porosity, and density, (iv) greater chemical homogeneity, or (v) greater conductivity compared with the common oxides (10, 28, 32, 34).

A variety of microorganisms, including heterotrophic bacteria, prosthecate bacteria, sheathed bacteria, fungi, algae, and their synergistic mixtures, can effect the conversion of soluble manganese to solid manganese oxides (5, 20, 23, 24). Microbial manganese oxidation mechanisms may be either direct or indirect. The direct mechanisms have been classified as either (i) enzymic catalysis or (ii) specific binding by cell-associated materials which enhance autooxidation (23). Indirect mechanisms refer to microbially promoted changes in oxidizing conditions in the cell's microenvironment that lead to nonbiological oxidation of Mn^{2+} .

The type of oxide formed can vary according to the type of microorganism and with changes in chemical, physical, and growth conditions of cultures. *Bacillus* sp. spores were reported to form hausmannite (Mn_3O_4) at pH 7.5 and a high

Mn^{2+} concentration ($>30 \mu M$), but when Mn^{2+} levels dropped below $30 \mu M$, manganite (γ - $MnOOH$) was formed (19). Chukhrov and colleagues (3) reported that *Metallogenium* sp. catalyzed the deposition of the disordered oxide vernadite (δ - MnO_2) when Mn^{2+} at up to 1 mM was present. The failure to isolate *Metallogenium* sp. in pure culture has led to doubts about its existence. The most common view is that the particles are not living forms themselves but result from the activity of microorganisms (6, 13, 20). These *Metallogenium* structures are particularly frequent in association with manganese-oxidizing fungi (35) and seem to result from manganese oxidation mediated by exopolymers produced by the fungus (6). In many reports, evidence of microbial involvement is inferred when dissolved manganese is rapidly removed to the biological particulate phase (7, 15).

The nonbiological oxidation of Mn^{2+} to MnO_2 in the presence of oxygen is thought to be a two-step process (17). The initial oxidation product is a metastable hausmannite, feitknechtite, or manganite, depending on temperature and pH (16, 22). Theoretically, a disproportionation reaction then forms δ - MnO_2 as the reaction ages. These reactions depend on ill-defined physicochemical conditions, often taking months or years to occur, particularly in the final stage of δ - MnO_2 formation (15, 17, 21). The chemical oxidation of manganous salts $MnCl_2$ and $MnSO_4$ by alkali metal chlorates was found to form γ - MnO_2 and α - MnO_2 , respectively (9).

The identification of manganese oxides often presents problems because of their complex structures. The most common method of analysis is X-ray diffraction. However, a number of phases, particularly the highly disordered, amorphous, and finely particulate species, are difficult to identify with any certainty by this method. Recently, it was shown that Fourier transform infrared (FTIR) spectroscopy could distinguish between most phases and show whether impurities were present (11, 27). Potter and Rossman (27) found infrared spectroscopy more reliable than X-ray diffraction when applied to disordered and finely divided samples.

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Most studies of biological manganese oxidation deal with descriptions of the formation of natural deposits, characterization of the associated microorganisms, or mechanistic studies. Up to now, very little work has been done on the possibility of using microorganisms to form disordered manganese oxides for battery use. The major reason for this lack of interest has been the low rates of biological manganese oxide formation. A rate-limiting factor in microbial manganese oxide synthesis is the inhibition of the microbes by high concentrations of manganous ion in batch culture.

In the work reported here, the effects of microbes on the formation of disordered manganese oxides have been examined at high manganous ion concentrations (1 to 5 g of Mn^{2+} /liter between pHs 6 and 7). Mixed cultures of microalgae and bacteria were used, and higher rates of oxide bioformation were achieved than have hitherto been recorded.

MATERIALS AND METHODS

Algal cultures. (i) Isolation and growth. An acid-tolerant microalga, *Chlamydomonas* sp., was isolated in nonaxenic culture from near a tailings dam at Mary Kathleen Mine, North Queensland, during the rehabilitation of the mine site. The alga was enriched from a mat of growth surrounding a drainage ditch containing water with approximately 8 mg of Mn^{2+} per liter at pH 2.1 and E_h 557 mV. The growth medium, an artificial mine water, was (per liter) 1.2 g of $(\text{NH}_4)_2\text{SO}_4$, 0.2 g of KH_2PO_4 , 0.2 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 mg of H_3BO_3 , 1.0 mg of $(\text{NH}_4)_6\text{MoO}_4 \cdot 4\text{H}_2\text{O}$, 15.8 mg of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.1 mg of ZnCl_2 , 0.3 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3 g of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, and 0.02 μg of biotin in 0.01 M H_2SO_4 (pH 2). The pH for growth and the microscopic appearance suggested that this species was *Chlamydomonas acidophilus*. A light (18-h)-dark (6-h) cycle was provided with Gro-lux fluorescent lamps. Cultures were aerated at approximately 2 liters/min.

(ii) Oxidation experiments. The algae were grown in a 2-liter glass reactor to an A_{310} of 1.0 in a cuvette with a 3-cm light path measured by a Shimadzu UV-160 spectrophotometer. Algal biomass was 5.7 g (dry weight) per liter.

(iii) Alga-stimulated manganese oxide deposition. Cells were harvested with a bench centrifuge at $500 \times g$ from 100 ml of an algal culture in log-phase growth. Each pellet was suspended in tap water with different concentrations of dissolved $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (0 to 100 g/liter) and urea (0 to 50 g/liter) and adjusted to pH 6.5. These cultures were transferred to 500-ml conical flasks that were then plugged with cotton wool, and the cultures were incubated in a static condition for 6 weeks at 30°C. The brown-black residue formed at the end of the incubation period was recovered by filtration and washed several times with dilute H_2SO_4 (0.03 M) and distilled water.

Bacterial isolation. Bacteria were isolated from a 10-ml aliquot of algal culture added to a 100-ml sterile bacterial growth medium containing 1 g of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.2 g of peptone, 0.1 g of yeast extract, and a salts mixture [50 mg of KH_2PO_4 , 100 mg of CaCl_2 , 200 mg of NaCl , 100 mg of $(\text{NH}_4)_2\text{SO}_4$, and 20 mg of MgSO_4 per liter]. This medium was incubated for 3 days at 30°C on an orbital shaker at 100 rpm. The resulting growth was termed primary mixed (crude) bacterial culture. Samples were streaked on solid medium (the growth medium plus 2% [wt/vol] agar). Single colonies were restreaked and subcultured until three different pure colonies were obtained. These isolates were inoculated into

the bacterial growth medium and incubated for 2 days. They were termed pure culture inocula.

The pure cultures were tested for oxide formation in oxidizing media with two different concentrations of manganese (0.5 or 5.0 g of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.25 g of peptone, and 0.25 g of yeast extract per liter). Aliquots were taken weekly for 5 weeks to determine the rate of oxide formation. The bacteria were Gram stained, and the best oxidizer was identified by using a Roche (Oxi-ferm tube) II analytical system.

Mn^{2+} removal from solution and oxide formation in algal-bacterial bioreactors. Bioreactors were constructed from 500-ml glass tubes (4-cm diameter by 42-cm height) with one end drawn to a 5-mm-diameter aperture. As a control (i.e., no added bacteria), 400 ml of algal culture was adjusted to pH 6.5. The algae sedimented at this pH and the 300 ml of supernatant were discarded, while the remainder was added to a glass tube containing 1 ml of Antifoam C emulsion (Sigma; 3% [wt/vol] solution) and autoclaved. For the experimental set up, the sedimented algae were added to a sterile bioreactor containing a filter-sterilized solution of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (15 g/liter), urea (10 g/liter), peptone (0.8 g/liter), and a salts mixture [50 mg of KH_2PO_4 , 100 mg of CaCl_2 , 20 mg of $\text{MgSO}_4 \cdot \text{H}_2\text{O}$, 100 mg of $(\text{NH}_4)_2\text{SO}_4$, and 200 mg of NaCl per liter] brought to a volume of 400 ml with distilled water and adjusted to pH 6.5.

The bioreactors were incubated at 30°C and aerated with filter-sterilized air at 2 liters/min. The volume was maintained at 400 ml by aseptic addition of sterile distilled water. Mn^{2+} in solution and the pH were measured for 4 weeks, after which the presence of oxide was determined with benzidine acetate reagent.

Experiments to determine individual effects of bacteria and algae in bioreactors. Eleven bioreactors were set up as 300-ml cultures. Eight separate sterile reactors had purified bacteria and combinations thereof added to autoclaved algae. $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (15 g/liter), urea (10 g/liter), peptone (0.8 g/liter), and a salts mixture [50 mg of KH_2PO_4 , 100 mg of CaCl_2 , 20 mg of $\text{MgSO}_4 \cdot \text{H}_2\text{O}$, 100 mg of $(\text{NH}_4)_2\text{SO}_4$, and 200 mg of NaCl per liter] were dissolved in distilled water, adjusted to pH 6.5, and sterilized. In control reactors, the bacterial inoculum or the algal addition was replaced by distilled water. All crude and pure bacterial incubates were aerated at 30°C and maintained at 300 ml by aseptic addition of sterile distilled water.

Effect of varying urea and algal biomass in bioreactors. Separate 300-ml sterile bioreactors with added mixed crude bacterial cultures were used. The urea concentrations were 0, 1, 5, 10, 15, 20, and 30 g/liter. Algal dry weight concentrations were 0, 2.1, 4.3, 5.7, 8.6, and 17.1 g/liter. The final precipitates were collected, washed, and weighed, and the manganese content was assessed.

Scale-up of bioreactors. The standard 300-ml algal bioreactor was tested on a larger scale (15 liters) to determine if type and yield of product altered. Fifteen liters of grown algae were adjusted to pH 6.5, concentrated by decantation to 4 liters, and added to a 20-liter vessel containing Antifoam C (30 ml of 3% [wt/vol] solution). $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (105 g), urea (105 g), peptone (12 g), and medium salts mixture were dissolved in 10.5 liters of distilled water, adjusted to pH 6.5, filter sterilized, and added to the vessel. The inoculum was 500 ml of the mixed crude bacterial culture. The reactor was aerated (2 liters/min) at 30°C in the dark.

Manganese oxide identification. (i) Preparation. Initial detection of microbially formed manganese oxide was by Feigl's benzidine spot test (8). The dark brown or black

FIG. 1. FTIR spectrum of oxide produced in algal culture, which is consistent with that of manganite. Arrows indicate typical manganite peaks.

solids produced in bioreactors were washed once in distilled water and then twice in 0.03 M H_2SO_4 (pH 1.5) to remove as much of the contaminating Mn^{2+} and other inorganic and organic compounds as possible. The oxides were then rinsed with distilled water until filtrates were acid free.

(ii) **Analyses.** FTIR spectroscopy (Nicolet MX-1E spectrometer) was used to tentatively characterize the oxides by comparison with a range of standard oxides. FTIR spectroscopy technique for characterization of manganese oxides is well documented (11, 27, 30).

X-ray diffraction (Siemens diffractometer) was also used for correlations with known oxides.

Electron microscopy and selected area electron diffraction examinations were made with a Hitachi H-7000 transmission electron microscope. Samples were prepared by transferring a drop of an aqueous oxide suspension to a Formvar-coated grid and allowing the drop to dry in air before examining it.

Chemical analyses. Soluble manganese (Mn(II)) was determined by using atomic absorption spectroscopy (Varian AA-1475). Total manganese content in oxide precipitates was determined by fully digesting a 20-mg sample of the oxide precipitate with 10 ml of boiling concentrated HCl, diluting it to 200 ml, and measuring the soluble manganese content. The O/Mn ratio was determined by the method of Murray et al. (21). Evaluation of the oxidizing capacity of the oxide was made by the Drottschmann's hydrazine consumption method as cited by Takahashi (31). The rate of manganese oxide formation was measured weekly, as described previously (14). Chemical fractionation of the alga into polysaccharide and cell solubles was performed with phenol-acetic acid as described by Strong et al. (29).

RESULTS AND DISCUSSION

Alga-stimulated manganite formation. When cultures of the nonaxenic acid-tolerant microalgae, a *Chlamydomonas* sp., were incubated in a static condition in the presence of urea and manganous sulfate near neutral pH, a dark brown precipitate formed which was identified by FTIR spectroscopy as manganite ($\gamma\text{-MnOOH}$) (Fig. 1). Urea, originally added as a nutrient for algal growth, was later found to aid in promoting biological manganese oxidation.

This manganite precipitate did not form unless the alga

FIG. 2. Time course of Mn^{2+} removal from solution and pH in an algal-bacterial bioreactor (control autoclaved).

and bacteria were present. Large numbers of bacteria grew in these cultures. Because the algal component in the presence of the primary mixed bacteria was still effective after autoclaving, it was assumed that the biological catalyst was bacterial, with participation of a heat-stable algal component. Larger amounts of oxide formed with increasing initial manganous ion concentrations, reaching an optimum around 225 mM. In the absence of urea, oxide formation was variable and the final pH of cultures was 5.74 ± 0.44 , whereas in the presence of 0.17 to 0.83 M urea, oxide precipitation was pronounced and the final pH was 6.76 ± 0.11 . It was inferred that an algal-dependent bacterial reaction was taking place in which urea or its breakdown products maintained favorable oxidation conditions.

Bacterial isolations. Three bacterial strains were isolated from the crude enrichment algal cultures. All were rods (0.5 to 3 μm), two were gram negative, and one was gram positive. Their colonies ranged from light creamy brown to dark brown or black, and they formed 5 ± 0.3 mg of Mn(IV) oxide per liter/week/10 mg of dry cells in solutions of 3 to 30 mM $\text{MnSO}_4 \cdot \text{H}_2\text{O}$. All were capable of growth and oxidation at Mn^{2+} concentrations greater than 30 mM on solid media. The colony morphology of each was constant on a particular medium but tended to change when either the Mn^{2+} or the nutrient concentrations were varied. Bacterial growth was also possible with only trace amounts of manganese present in the medium. The highest rates of oxidation of manganese were exhibited by a gram-negative aerobic rod identified as *Pseudomonas* sp. strain MK-1.

Mn^{2+} removal from solution and oxide formation in algal-bacterial bioreactors. Figure 2 shows the events in a mixed algal-bacterial culture. The Mn^{2+} removal from solution was four times less in autoclaved controls than in the test solution, in which Mn^{2+} was removed at a rate of 0.83 g/liter/week. The bacterial inoculum was derived by enrichment of the bacteria associated with the nonaxenic algal cultures. The test reactor contained an appreciable black precipitate after 1 month of culture, whereas the sterile control retained the unchanged green color of the algae. This result confirmed the involvement of microbes in both Mn^{2+} removal from solution and oxide formation. Furthermore, the neutral pH throughout the incubation and the comparatively low temperatures suggested a biological rather than a simple chemical oxidation phenomenon.

Effects of bacteria and algae in bioreactors. Table 1 shows

TABLE 1. Effects of bacteria and algae on bioformation of manganese oxides^a

Condition no.	Bacterial inoculum ^b	Algal ^c addition	Amt (g) of:		
			Mn ²⁺ removed from solution ^d	Impure precipitate ^e	Oxide manganese ^f
1	Pure MK 1	Sterile	1.12	1.11	0.38
2	Pure MK 2	Sterile	0.73	0.69	0.24
3	Pure MK 3	Sterile	0.29		
4	MK 1 + MK 2	Sterile	0.86	0.92	0.29
5	MK 1 + MK 3	Sterile	0.76	0.39	0.07
6	MK 2 + MK 3	Sterile	0.48		
7	MK 1 + MK 2 + MK 3	Sterile	0.59	0.24	0.05
8	Mixed enrichment	Sterile	0.60	0.96	0.29
9	Mixed enrichment	Nonsterile	0.74	0.99	0.36
10	Mixed enrichment	None	0.20		
11	None	Sterile	0.24		

^a Bioreactors were 300 ml in volume, and incubations were for 4 weeks. The results were generally reproducible, though variations of up to 20% can occur in amounts of Mn²⁺ removed from solution, yields, and manganese contents of precipitates.

^b Mixed bacterial enrichments were derived from cultures of algae.

^c Nonaxenic algal cultures were grown at pH 2 raised to pH 6.5 and were autoclaved to sterilize them.

^d Soluble Mn²⁺ was taken up from solution by cell adsorption or precipitation.

^e Black oxide product containing cell residues was washed rigorously with 0.03 M H₂SO₄ at 20°C to remove acid-soluble compounds (i.e., adsorbed Mn²⁺ and cells). Impure precipitates at less than 0.1 g are not shown because they were primarily organic remains.

^f Total Mn(II) in impure precipitates (from HCl digests). Less than 0.01 g was considered insignificant.

the effects of bacteria and microalgae on bioformation of manganese oxides. Isolate MK-1 was clearly the most active bacterium in the formation of a manganese precipitate in the presence of algae, while MK-3 was unable to form an oxide in pure culture. Remixing with strains MK-2 and MK-3 reduced oxide production by MK-1. The addition of all three bacteria resulted in the formation of an even lower amount of manganese precipitate because of competition between the three and because of the higher proportions of MK-2 and MK-3 relative to MK-1. The quality and structural characteristics of the manganese precipitates produced by the pure bacteria were not assessed and will be studied more closely in future work.

The crude enrichment (mixed culture) with either sterile or nonsterile algae (conditions 8 and 9) formed oxide at similar rates, showing that *Chlamydomonas* cells had a physical or chemical role rather than an active metabolic function. When the alga was omitted, the crude mixed bacterial culture formed only very small amounts of oxide (condition 10). Similarly, if only sterile algae were used (condition 11), no oxide formed. Therefore both microalgae and bacteria were essential for oxide formation.

Effects of urea and algal biomass on bacterial formation of

manganese oxides. Figures 3a and b show the effects of urea concentration and the amount of algal biomass on oxide production. Urea was optimal at 10 g/liter (167 mM), while algal biomass was 5.7 g (dry weight) per liter. Urea may behave as a nutrient which by way of urease cleavage provides a source of nitrogen or carbon, though this has yet to be proved. Excess urea (>10 g/liter) probably leads to metabolic inhibition from ammonia toxicity and protein denaturation.

While the role of the *Chlamydomonas* sp. is obligatory, the actual mechanism of action is not obvious. Preliminary fractionation of the alga with phenol-acetic acid solutions showed that oxide-enhancing activity was associated with the algal cell solids rather than soluble materials. The algal cell is very durable, and microscopically there are no obvious differences between the freshly grown material and that which has been autoclaved or subjected to nonphysiological pH in the bioreactor. A surface property may be involved, as has been demonstrated with *Chlorella* sp. by Darnall et al. (4).

The addition of algae, urea, and Mn²⁺ in the absence of bacteria resulted in some removal of urea and Mn²⁺ from solution (about a quarter of the removal with bacteria) with

FIG. 3. Effects of various urea concentrations (a) and algal biomasses (b) on bacterially formed manganese oxides in algal-bacterial bioreactors.

FIG. 4. Effect of algal sterility on FTIR spectra of manganese oxide products formed in algal-bacterial bioreactors. (a) Nonsterile algae; (b) autoclaved algae.

no oxide precipitation. This suggests the formation of an alga-urea- Mn^{2+} complex providing a site for bacterial oxidation.

Effect of autoclaving algae. The yield of manganese oxides from cultures using viable algae was only slightly superior to that from cultures with autoclaved algae. The FTIR spectra of semipure oxides from these two conditions were almost identical (Fig. 4). The oxide from the viable algal reaction had a slightly more intense Mn-O peak. Both had a broad major peak at 570 wavenumbers and closely resembled a known disordered MnO_2 standard.

Precursor role of manganite in microbiological formation of disordered manganese oxide. Oxide samples were taken after 10, 20, and 30 days of culture and analyzed by FTIR spectroscopy (Fig. 5). The 10-day sample shows the presence of manganite, with characteristic O-H bands at around 2,690 and 2,048 wavenumbers along with those at 594 and 522 wavenumbers. The 20-day sample shows that most of the manganite peaks had disappeared, and after 30 days, no manganite remained. It was replaced by an intense, disordered MnO_2 peak at around 570 wavenumbers. The result indicated that manganite is possibly a precursor in microbial formation of disordered manganese oxide. This was a preliminary finding and requires further investigations for confirmation.

Under conditions of near-neutral pH and 30°C, chemical conversion of MnOOH to MnO_2 within a period of days is highly unlikely. It would take at least a number of months and possibly even years, since metastable $\gamma\text{-MnOOH}$ can exist unchanged for some time. Furthermore, if there was an abiotic conversion, the result would be a disproportionation reaction: $2\text{MnOOH} + 2\text{H}^+ \rightarrow \text{MnO}_2 + \text{Mn}^{2+} + 2\text{H}_2\text{O}$. As the mole ratio is 2:1, the yield of MnO_2 would be falling instead of increasing, and Mn^{2+} would be released into solution rather than being removed over the 30 days of incubation.

Large-scale algal-bacterial reactors. An algal-bacterial bioreactor having a 15-liter operating volume was run. The kinetic data and other results are shown in Table 2. While there were some reductions in yield per volume and rates, the structure and quality of the oxide did not change. The rate of Mn^{2+} removal from solution, the manganese content

FIG. 5. FTIR spectra of manganese oxide from an algal-bacterial reactor at different incubation times. Arrows indicate manganite peaks.

of the oxide, and the impure oxide yield were equivalent to that from a small 300-ml bioreactor.

The oxidation state of the manganese was 3.84 (i.e., a manganese oxide as $\text{MnO}_{1.92}$). The hydrazine index, a measure of the oxidizing capacity of the manganese oxide, was 28.4, indicating possible battery activity, although in the low

TABLE 2. Product analysis and characterization of large-scale manganese oxide formation in a 15-liter algal-bacterial bioreactor^a

Parameter investigated	Result
Rate of Mn^{2+} removal from solution	0.36 g/liter/wk
Total Mn^{2+} removed from solution ^b	21.6 g
Impure oxide yield ^c	22.3 g
Rate of impure oxide formation	0.37 g/liter/wk
Manganese content in oxide ^d	30.5%
Mn(IV) content in oxide	6.80 g
Conversion of Mn(II) to Mn(IV)	31.5%
Oxidation state (O/Mn ratio)	1.92
Hydrazine index	28.4

^a Incubation was for 4 weeks.

^b Soluble Mn^{2+} was taken up from solution by cell adsorption or precipitation.

^c Black oxide product containing cell residues was washed rigorously with 0.03 M H_2SO_4 at 20°C to remove acid-soluble compounds (i.e., adsorbed Mn^{2+} and cells).

^d Total Mn(II) in impure precipitates (from HCl digests).

FIG. 6. X-ray diffraction pattern of the manganese oxide formed in an algal bioreactor. Note the broad peaks at 0.142, 0.164, 0.212, and 0.242 nm.

range. The FTIR spectrum was superimposable on those of oxides recovered from the smaller bioreactors, showing a prominent MnO_2 peak at 570 wavenumbers. X-ray diffraction confirmed the oxide as disordered $\gamma\text{-MnO}_2$ with d-spacings at 0.142, 0.164, 0.212, and 0.242 nm (Fig. 6). The broadness of the X-ray peaks indicated the amorphous and disordered nature of the oxide. Electron diffraction also showed that the $\gamma\text{-MnO}_2$ phase was predominant in both untreated and acid-washed products (results not shown).

General discussion. Previous quantitative studies on microbial manganese oxidation have been done at very low manganous ion concentrations (in the milligrams-per-liter range) unsuitable for industrial scale. In the present work, the algal presence seems to have alleviated the inhibitory effect of high concentrations of soluble manganese, making it possible to carry out oxidation reactions at Mn^{2+} concentrations in the grams-per-liter range. It seems probable, particularly in the absence of convincing evidence that the alga contributes nutrient for the bacteria, that the surface properties of this acid-tolerant *Chlamydomonas* sp. are involved in manganous ion tolerance. Histochemical-staining reactions show an abundance of extracellular polysaccharide on the alga. An anionic polymeric mucilage could reduce metal ion toxicity by sequestering Mn^{2+} . Retention of the manganous ion-protective function of the alga after heat sterilization suggests that a thermostable polysaccharide may be the key component.

At present, the major drawback of microbially formed manganese oxides for use in dry-cell batteries is the presence of biological impurities. The Mn(IV) content in the final oxide was relatively low compared with chemically and electrolytically formed and mined disordered oxides. Battery manganese oxides usually have around 50% Mn(IV) , with battery activity directly related to MnO_2 content. The biological impurities (ca. 20% [wt/vol]) which constitute residual algal, bacterial, and cell debris are difficult to remove. Further studies are under way to devise a technique for purifying these oxides.

Conclusions. The biotechnology for microbiological formation of disordered manganese oxides has been demonstrated. The system depends on a viable bacterium (a *Pseudomonas* species) and a microalga (a *Chlamydomonas* species). The

presence of both organisms was needed to obtain quantitatively significant oxide formation. The alga enhanced oxide formation even after metabolic inactivation by autoclaving. Manganite was seen to form as a precursor in month-old cultures, while the final product was a disordered manganese oxide. The identity of the disordered oxide was confirmed by FTIR spectroscopy, X-ray and electron diffraction correlations, and a variety of chemical determinations.

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