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Sequestration of Oxyanions of V(V), Mo(VI), and W(VI) Enhanced through Enzymatic Formation of Fungal Manganese Oxides

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Abstract: Biogenic Mn oxides (BMOs) have become captivating with regard to elemental sequestration, especially at circumneutral pH conditions. The interaction of BMOs with oxyanions, such as vanadate (V), molybdate (VI), and tungstate (VI), remains uncertain. This study examined the sequestration of V(V), Mo(VI), and W(VI) (up to ~1 mM) by BMOs formed by the Mn(II)-oxidizing fungus, Acremonium strictum KR21-2. When A. strictum KR21-2 was incubated in liquid cultures containing either Mo(VI) or W(VI) with soluble Mn$^{2+}$, the oxyanions were sequestered in parallel with enzymatic Mn(II) oxidation with the maximum capacities of 8.8 mol% and 28.8 mol% (relative to solid Mn), respectively. More than 200 µM V(V) showed an inhibitory effect on growth and Mn(II) oxidizing ability. Sequestration experiments using preformed primary BMOs that maintained the enzymatic Mn(II) oxidizing activity, with and without exogenous Mn$^{2+}$, demonstrated the ongoing BMO deposition in the presence of absorbent oxyanions provided a higher sequestration capacity than the preformed BMOs. X-ray diffraction displayed a larger decline of the peak arising from (001) basal reflection of turbostratic birnessite with increasing sequestration capacity. The results presented herein increase our understanding of the role of ongoing BMO formation in sequestration processes for oxyanion species at circumneutral pH conditions.

Keywords: manganese oxide; biomineralization; birnessite; oxyanion; Mn(II)-oxidizing fungus; vanadate (V); molybdate (VI); tungstate (VI)

1. Introduction

Anthropogenic activities have rapidly increased the emission of various elements, including oxyanion species, such as V(V) [1], Mo(VI) [2], and W(VI) [3]. Excessive concentrations of these elements are toxic to humans and other organisms [4–7]. Therefore, efficient remediation systems are required to maintain a clean environment worldwide [8,9]. These oxyanions, V(V), Mo(VI), and W(VI), readily accumulate natural manganese oxide phases [10–12]. Therefore, manganese oxide may be a potent scavenger for these oxyanions.

Biogenic nanosized metal oxide particles, such as Fe and Mn oxides act as scavengers for various inorganic elements (ions) in natural environments. Consequently, the biogenic metal oxide formation (biomineralization) through biologically mediated redox reactions is one of the most interesting processes for developing an efficient element remediation system [13,14]. Among the biomineralization processes frequently found in natural environments, biogenic Mn oxides (BMOs) formation processes by fungi and bacteria are captivating because the sequestration ability of the BMO is very high, and they subsequently determine the fate of a variety of elements [15–18]. Both in bacterial and fungal...
BMO formation, enzymatic Mn(II) oxidation with O₂ (as an electron acceptor) by multicomponent oxidase (MCO) is considered to be a key process that readily produces BMOs [19–21]. MCOs that catalyze BMO formation were frequently identified in bacterial Mn(II)-oxidizers, such as Bacillus sp. strains, SG-1 and PL-12, Lepthothrix discophora, Pseudomonas putida, and Pedomicrobium sp. ACM 3067 [22]. Among fungal Mn(II)-oxidizers, Acremonium strictum KR21-2 was isolated from an Mn-oxide coated pebble surface in Kikukawa River, Shizuoka, Japan [19,23]. It has been well demonstrated to secrete an MCO-type Mn(II)-oxidizing enzyme to precipitate BMOs. Our recent research on A. strictum KR21-2 (used as a model Mn(II)-oxidizing micro-organisms) showed that the corresponding enzyme is MCO (named Mco1), assigned to a bilirubin oxidase [21]. The heterologous expression of the gene encoding Mco1 in the methylotrophic yeast Pichia pastoris successively produces the recombinant Mco1 that oxidizes Mn(II) to form BMOs at an initial 0.6 mM Mn(II) within 3 h [21]. This result shows that BMO formation readily proceeds from ~mM level Mn(II) when the enzyme effectively catalyzes Mn(II) oxidation reactions.

Extensive studies on BMOs have investigated the cationic metal sequestration ability relating to their mineralogical characteristics [24–26]. BMOs (fungal and bacterial) are primarily layered analogously to vernadite, a nanostructured and turbostratic variety of birnessite. The crystallite has a domain dimension of ~10 nm in the layer plane [27], providing a high extent of the structural edge sites on which surface complexation and redox reactions can proceed effectively. In addition, the negatively charged octahedral vacancy sites (up to ~30%) in the birnessite sheet structure of BMOs [27] act as effective sorption sites for various cations. Previous studies [28–32] have demonstrated more effective sequestration abilities of BMOs for different metal cations than chemically synthesized Mn oxides. In contrast, few studies have been conducted on the sequestration ability of BMOs (fungal and bacterial) for oxyanions. Therefore, the applicability of BMO to remove (recover) toxic oxyanions remains uncertain.

A previous study found that newly formed BMOs in the cultivation of A. strictum KR21-2 effectively oxidize exogenous Mn²⁺ to form different BMO phases because the enzymatic activity is stably maintained in the primary BMO phase [33]. This process strongly affects the mineralogical feature of the resultant BMO phase when the BMO formation progresses under the coexistence of the guest elements (ions) sequestered into the BMO phase thus formed [32,34,35]. The crystal structure of BMOs is typically turbostratic birnessite, when no associated cation coexists [34]. In contrast, coexisting Ba²⁺ at a Ba²⁺/Mn²⁺ > 1 results in well-laminated birnessite, where preferential Ba²⁺ incorporation into the interlayer occurs irreversibly [34]. Coexisting Zn²⁺ and Co²⁺ during Mn oxidation by fungal BMOs provide mixed Zn/Mn and Co/Mn oxide phases of woodruffite (ZnMn⁴⁺,O₇,2H₂O) [32] and asbolane [35]. Such mineralogical variation of the resultant BMO phases is important not only for biogeochemical association in Mn oxide phases but also for metal resource recovery and the remediation of water contaminated with toxic elements. However, the effect of coexisting V(V), Mo(VI), and W(VI) on enzymatic Mn(II) oxidation (BMO formation) and the subsequent sequestration ability remains unknown. We hypothesize that the mineralogical feature of BMO is influenced by the coexistence of these oxyanions when enzymatic BMO formations continuously progress, and subsequently, their sequestration ability should be strongly affected.

This study aims to clarify the ability of the Mn(II)-oxidizing fungus, Acremonium strictum KR21-2 [19–21], to sequester V(V), Mo(VI), and W(VI) on enzymatic Mn(II) oxidation (BMO formation) and the subsequent sequestration ability remains unknown. We hypothesize that the mineralogical feature of BMO is influenced by the coexistence of these oxyanions when enzymatic BMO formations continuously progress, and subsequently, their sequestration ability should be strongly affected.

This study aims to clarify the ability of the Mn(II)-oxidizing fungus, Acremonium strictum KR21-2 [19–21], to sequester V(V), Mo(VI), and W(VI) through enzymatic BMO formation. For this purpose, we examined the sequestration experiments for the oxyanions during the incubation of A. strictum KR21-2 in batch culture experiments with 1 mM Mn(II) and each of V(V), Mo(VI), and W(VI) (20–1000 µM). The results show that more than 200 µM V(V) exhibited an inhibitory effect on growth and Mn(II) oxidizing ability. At the same time, enzymatic BMO formation and concomitant Mo(VI) and W(VI) sequestrations were observed even at 1000 µM Mo(VI) (up to 8.8 mol% relative to solid Mn) and W(VI) (up to 28.8 mol% W). These results suggest that A. strictum KR21-2 is a potent micro-organism to recover Mo(VI) and W(VI) from contaminated water through BMO formation. We
also conduct sequestration experiments for oxyanions using newly formed (preformed) BMOs under the condition where exogenous Mn$^{2+}$ was enzymatically oxidized and under that where it was not. The results demonstrate that the ongoing BMO formation readily facilitates the sequestration ability for coexisting V(V), Mo(VI), and W(VI) (normalized to oxide Mn). X-ray diffraction measurements show mineralogical alteration with a decline in the peak intensity of (001) basal reflection, when BMO is formed from exogenous Mn(II) with coexisting oxyanions, suggesting that the coexisting oxyanions prevent the sheet stacking of BMO.

2. Materials and Methods


All chemical reagents used in this study were of analytical grade and purchased from FUJIFILM Wako Chemical Co. (Osaka, Japan) except for the yeast extract (36802-16), purchased from Nacalai Tesque, Inc. (Kyoto, Japan). To evaluate the influence of coexisting V(V), Mo(VI), and W(VI) on the growth and Mn(II)-oxidizing ability, a conidium suspension ($1 \times 10^5$ conidia mL$^{-1}$) of A. strictum KR21-2 was incubated in HAY liquid medium (20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) buffer adjusted at pH 7.0 with NaOH) [36,37] with MnSO$_4$ (1 mM) and either Na$_3$VO$_4$, Na$_2$MoO$_4$, or Na$_2$WO$_4$ (up to 1 mM) at 25 °C for 72 h on a reciprocal shaker, set at 105 strokes min$^{-1}$ (NR-10, Taitec, Nagoya, Aichi, Japan). After incubation for 72 h, dissolved Mn(II) and either V(V), Mo(VI), or W(VI) in the supernatant were measured by a Varian 730-ES inductively coupled plasma-atomic emission spectrometer (ICP-AES) (Agilent Inc., Santa Clara, CA, USA) to determine the Mn(II) oxidizing and oxyanion sequestration abilities. Growth was measured as a fungal mass weight as previously described [29,32,37]. In some experiments, the supernatants were sampled at the appropriate times and used for analyzing dissolved species to measure the time courses of Mn(II) oxidation and sequestration of oxyanions during the cultivation [36].

2.2. Sequestration of Oxyanions by Newly Formed BMOs with and without Exogenous Mn$^{2+}$

After incubating A. strictum KR21-2 in 1 mM Mn$^{2+}$-supplemented HAY medium (pH 7.0) for 72 h, the solid phase (biogenic Mn oxides plus fungal mycelia; hereafter designated “newly formed BMO” [37]) was harvested and washed three times with 20 mM HEPES buffer (pH 7.0) as previously described [37]. Newly formed BMOs (1 mM as Mn) were treated in 20 mM HEPES (pH 7.0) solutions containing 0.08–0.8 mM V(V) with and without exogenous Mn$^{2+}$ (1 mM MnSO$_4$) at 25 °C for 24 h on a reciprocal shaker, set at 105 strokes min$^{-1}$. Dissolved V(V) and Mn(II) in the supernatant were determined by ICP-AES. After 24 h of treatment, the residual solids were subjected to a two-step extraction protocol using aqueous 10 mM CuSO$_4$ (pH of 4.8) and 50 mM hydroxylamine hydrochloride for speciation of Mn$^{2+}$ sequestered by the BMOs, as previously described [29,32,37]. This extraction sequence commonly serves for fractionating exchangeable (sorbed) Mn(II) and reducible (oxidized) Mn from BMOs [38–40].

Sequestration experiments for oxyanions were conducted to evaluate the effect of the enzymatic Mn(II) oxidation process by newly formed BMOs on the sequestration ability under three experiment procedures listed in Table 1, where a and b in a term “BMO$_a$/ExMn$_b$” denote the concentrations of primary BMO (mM as Mn) and exogenous Mn$^{2+}$ (mM), respectively, used in the sequestration experiments. The sequestration reactions were carried out aerobically for 24 h at 25 °C at 105 strokes min$^{-1}$. 
Table 1. Experimental conditions for sequestration experiments using newly formed BMOs with and without exogenous Mn$^{2+}$.

<table>
<thead>
<tr>
<th>Primary BMOs</th>
<th>Sequestration Process</th>
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<tr>
<td>BMO$<em>{2.0}$/ExMn$</em>{0.0}$</td>
<td>The newly formed BMO$^2$ (1 mM as Mn) was reacted with exogenous Mn$^{2+}$ (1 mM) to form additional BMO phases in 20 mM HEPES at 7.0 for 24 h. This primary BMO contained 2 mM Mn. V(V), Mo(VI), or W(VI) (up to ~1 mM) without exogenous Mn$^{2+}$ in 20 mM HEPES (at pH 7.0) for 24 h.</td>
</tr>
<tr>
<td>BMO$<em>{1.0}$/ExMn$</em>{1.0}$</td>
<td>Newly formed BMOs$^2$ (1 mM as Mn) V(V), Mo(VI), or W(VI) (up to ~1 mM) with 1 mM exogenous Mn$^{2+}$ in 20 mM HEPES (at pH 7.0) for 24 h.</td>
</tr>
<tr>
<td>BMO$<em>{0.5}$/ExMn$</em>{1.5}$</td>
<td>Newly formed BMOs$^2$ (0.5 mM as Mn) V(V), Mo(VI), or W(VI) (up to ~1 mM) with 1.5 mM exogenous Mn$^{2+}$ in 20 mM HEPES (at pH 7.0) for 24 h.</td>
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1 In the term “BMO$_a$/ExMn$_b$”, a and b denote the concentration of the primary BMO (mM as Mn) and exogenous Mn$^{2+}$ (mM), respectively. 2 Newly formed BMOs were formed through the cultivation of *Acremonium strictum* KR21-2 in HAY liquid media at pH 7.0 with MnSO$_4$ (0.5 or 1 mM) for 72 h.

All the sequestration and extraction experiments were conducted in triplicate ($n = 3$), and data in the figures and tables are shown as mean ± standard deviation.

2.3. XRD Measurements of BMOs after the Sequestration Experiments

XRD measurements were performed for the BMOs after the sequestration experiments. A Rint2500 diffractometer (Rigaku Co., Akishima, Tokyo, Japan) was operated with CuK$_\alpha$ radiation at 26 mA and 40 kV. BMO samples treated under the experimental conditions listed in Table 1 were harvested by a 100 µm nylon mesh (FALCON Cell Strainer 352360, Corning Inc., Corning, NY, USA), washed three times with Milli-Q water, and frozen at −60 °C. After lyophilization using an EYELA Freeze Dryer FD-1000 (Tokyo Rikakikai Co., LTD., Tokyo, Japan), lyophilized BMO samples were placed on a glass holder and scanned over a 2θ range of 5–70° at 1.0° min$^{-1}$ using a 0.02° step interval.

3. Results and Discussion


When the conidia suspension of *A. strictum* KR21-2 is incubated in HAY liquid culture media (20 mM HEPES at 7.0) with 1 mM MnSO$_4$, the concentration of dissolved Mn(II) abruptly decreases at 36–45 h incubation due to the enzymatic formation of insoluble Mn oxide [19–21,36]. When either ~20 µM V(V), Mo(VI), W(VI) or Cr(VI) coexisted during the incubation, *A. strictum* KR21-2 readily formed a BMO and subsequently dissolved Mn(II) (1 mM) completely (>99%) were converted to the solid phase Mn (Figure 1A–D). The solid phase Mn formed comprised ~20% exchangeable Mn and ~80% reducible Mn [28]. The concentrations of V(V), Mo(VI), and W(VI) were decreased synchronously with Mn oxide formation (Figure 1A–C) while dissolved Cr(VI) concentration remained unchanged throughout incubation (Figure 1D).
Figure 1. Sequestration of (A) V(V), (B) Mo(VI), (C) W(VI) (~20 µM) synchronized with biogenic Mn oxidation (~1 mM, and ~0.2 mM for W(VI)) during the incubation of *Acremonium strictum* KR21-2 in HAY liquid media (20 mM HEPES buffer at pH 7.0). No significant sequestration was observed when no Mn$^{2+}$ was supplemented. (D) Sequestration of Cr(VI) was negligible even when the biogenic Mn oxide was formed. Shadows indicate the timing of biogenic Mn oxide formation typically 34–46 h of the incubation. The error bars represent the standard deviation ($n = 3$).

There were no significant changes in dissolved V(VI), Mo(VI), and W(VI) during incubation when MnSO$_4$ was not supplemented (Figure 1A–C), indicating that the BMO formation mediated the sequestrations. Consequently, the sequestration on fungal hyphae (biomass) was negligible for all oxyanions used in this study. Sequestration efficiency was the highest for W(VI) at >99%, where the initial W(VI) at 22.0 ± 0.2 µM decreased to 0.06 ± 0.00 µM (Figure 1C). Sequestration efficiency of W(VI) at the initial concentration of 19.2 ± 1.0 µM was still high at 42% even when the MnSO$_4$-supplementation was lower at...
0.22 ± 0.00 mM (Figure 1C), where the molar ratio of sequestered W relative to solid Mn was deduced at 0.037 (mol/mol). Sequestration efficiency for V(V) at the initial concentration of 23.0 ± 0.6 μM was 62% with the V/Mn molar ratio of 0.013 (mol/mol) when 1.07 ± 0.02 mM Mn$^{2+}$ was supplemented (Figure 1A). For Mo(VI), 42% of the initial Mo(VI) at 22.6 ± 0.3 μM was sequestered with 1.09 ± 0.00 mM Mn$^{2+}$-supplementation (Figure 1B). Under the experimental condition, the sequestration affinity is W(VI) > V(V) > Mo(VI) >> Cr(VI). It should be noted that the HAY liquid culture medium contains 200 μM MgSO$_4$, 50 μM CaCl$_2$, 30 μM K$_2$HPO$_4$, and 0.2–1 mM MnSO$_4$ added as an exogenous Mn(II) supplement. Therefore, anionic species from such constituents in the liquid media should be potential competitors for sequestration on BMO. Thus, BMO favors W(VI), V(V), and Mo(VI) compared to SO$_4$$^{2-}$ and VPO$_4$$^{4-}$ (H$_2$PO$_4^-$). No significant sequestration of Cr(VI) by BMO (Figure 1D) was consistent with our previous study, which demonstrated a release of Cr(VI) due to the oxidation of Cr(III) being sorbed on BMOs [41].

To clear the influence of the coexisting oxyanions on the sequestration ability during the incubation of *A. strictum* KR21-2, we measured the sequestration efficiency of V(V), Mo(VI), and W(VI) concurrent with BMO formation with the initial concentrations of each oxyanion, up to ~1000 μM. The fungal growth (based on the fungal mass weight after 72 h of incubation) and the Mn oxidation ability (based on Mn conversion efficiency from aqueous to solid phase) were also monitored after 72 h of incubation. The increasing concentrations of V(V) slowed the growth rate of *A. strictum* KR21-2 with a relative fungal mass of only 10.7 ± 6.4% at 1.23 ± 0.02 mM V(V) (Figure 2A). *A. strictum* KR21-2 abruptly lost the Mn(II) oxidation ability (<12.1 ± 0.4%; Figure 2D) at the concentration of V(V) is above 239 ± 2 μM V(V). These results demonstrated that the adverse effect of V(V) on the growth and Mn oxidation ability of *A. strictum* KR21-2 is similar to heavy metal ions, such as Co$^{2+}$, Ni$^{2+}$, and Cd$^{2+}$.

Figure 2. Effects of coexisting (A,D,G) V(V), (B,E,H) Mo(VI), and (C,F,I) W(VI) on: (A–C) growth, (D–F) Mn(II) oxidizing ability, and (G–I) subsequent sequestration ability for oxyanions during the incubation of *Acremonium strictum* KR21-2 in HAY liquid media (20 mM HEPES buffer at pH 7.0). The error bars represent the standard deviation (n = 3).
Our previous studies have shown the growth inhibition at \( \geq 100 \, \mu M \) Co\(^{2+}\) [37], Ni\(^{2+}\) [29], and Cd\(^{2+}\) [29] (Zn\(^{2+}\) did not show the growth inhibition up to 1 mM [32]) and the complete loss of Mn(II) oxidizing ability at \( \geq 100 \, \mu M \) Co\(^{2+}\) [37], \( \geq 50 \, \mu M \) Zn\(^{2+}\) [32], \( \geq 30 \, \mu M \) Ni\(^{2+}\) [29], and \( \geq 50 \, \mu M \) Cd\(^{2+}\) [29]. Therefore, it seems that the adverse effect of V(V) on the growth and Mn(II) oxidizing ability is somewhat smaller than that of such heavy metal ions. The threshold of V(V) at \( \sim 200 \, \mu M \) (10,000 \( \mu g/L \) V) for Mn(II) oxidation obtained in this study (Figure 2D) is much higher than the dissolved concentration of dissolved V that is reported to be \( \sim 0.7 \, \mu g/L \) in river water and \( \sim 1.8 \, \mu g/L \) in seawater [8]. Thus, the in-situ remediation by the BMO formation process through the incubation of the Mn(II) oxidizing fungi is still applicable for remediating wastewater contaminated with V(V) below \( \sim 100 \, \mu M \) (500 \( \mu g/L \)).

In contrast to V(V), even when Mo(VI) and W(VI) were added up to 1.26 \( \pm \) 0.02 mM and 1.19 \( \pm \) 0.00 mM, respectively, the growth rate (\( > \)89% of the fungal mass base) and Mn(II) oxidation efficiency (\( >94\% \)) were readily maintained after 72 h of incubation (Figure 2B,C). Subsequently, sequestrations of Mo(VI) and W(VI) progressed concurrently with BMO formation, with the highest Mo(VI) and W(VI) at 0.088 \( \pm \) 0.003 (mol/mol) and 0.288 \( \pm \) 0.004 (mol/mol) as the molar ratios relative to solid Mn in the solid phases, respectively (Figure 2H,I). These results demonstrated the applicability of the fungal BMO formation process to recover those oxyanions from aqueous solutions. When 1 mM Mn\(^{2+}\) was used as an additive for fungal BMO formation, quantitative recovery (\( >93.8 \% \) of 0.02 mM) was achieved (Figure 2F).

### 3.2. Sequestrations of Oxyanions by Newly Formed BMOs with an Mn(II) Oxidizing Enzymatic Activity

The growth and subsequently Mn(II) oxidation ability are susceptible to inhibition by V(V) at \( >100 \, \mu M \) similar to heavy metal ions, such as Co\(^{2+}\), Ni\(^{2+}\), and Cd\(^{2+}\). Our previous studies demonstrated that when A. strictum KR21-2 is incubated in HAY liquid media with Mn\(^{2+}\), newly formed BMOs maintain the enzymatic activity to oxidize Mn\(^{2+}\) and readily convert soluble Mn\(^{2+}\) to Mn(III/IV) oxide even when Co\(^{2+}\), Ni\(^{2+}\), or Cd\(^{2+}\) coexist at the mM level [33]. To investigate the ability of newly formed BMOs to sequester V(V) and to oxidize Mn\(^{2+}\), the sequestration experiments for V(V) were conducted using the newly formed BMO (\( \sim 1 \, mM \) as Mn) with and without exogenous Mn\(^{2+}\) (\( \sim 1 \, mM \) as Mn\(\text{SO}_4\)) in 20 mM HEPES at pH 7.0. When newly formed BMOs were reacted with mixed solutions of exogenous Mn\(^{2+}\) (\( \sim 1 \, mM \)) and V(V) (80, 400, or 800 \( \mu M \)), the concentrations of Mn\(^{2+}\) disappeared within 4 h of the reaction (Figure 3A). At 24 h, the two-step extraction procedure displayed that the solid phase Mn comprised 82.9–83.6% reducible Mn (Figure 3B), which was the same as Mn in the primary BMO phase, indicating that newly formed BMO successfully oxidized exogenous Mn\(^{2+}\) even when V(V) coexisted at 800 \( \mu M \). The concentrations of dissolved V(V) decreased in parallel with soluble Mn\(^{2+}\) concentrations within 4 h of the reaction. Subsequently, they reached constant values (Figure 3A). The amounts of V(V) sequestered were considerably higher than that without exogenous Mn\(^{2+}\) (Figure 3C), indicating that the V(V) sequestration was facilitated by the ongoing formation of Mn oxide from soluble Mn\(^{2+}\). Interestingly, the sequestration amounts of V(V) normalized to reducible (oxidized) Mn tended to be higher in the case of exogenous Mn\(^{2+}\). The BMO phase formed from exogenous Mn\(^{2+}\) and coexisting V(V) may possess mineralogical characteristics (and consequently the V(V) sequestration ability) different from that of the primary BMO phase (formed without the coexisting V(V) before the V(V) sequestration experiments). For example, Ba\(^{2+}\) coexisting during enzymatic Mn(II) oxidation leads to a well-laminated birnessite structure of the resultant Mn oxide phase and consequently leads to the high irreversibility of Ba\(^{2+}\) sequestration [34]. Our previous studies also demonstrated that Zn\(^{2+}\) and Co\(^{2+}\) coexisting during enzymatic Mn(II) oxidation causes the formation of binary oxide minerals, such as woodruftite [32] and asbolane [35], respectively, subsequently increasing the sequestration efficiencies of these heavy metal ions through the enzymatic Mn(II) oxidation processes.
3.3. Sequestrations of Oxyanions through Enzymatic Mn(II) Oxidation

To evaluate the effect of the enzymatic BMO formation process on the sequestration of V(V), Mo(VI), and W(VI), we conducted sequestration experiments under the three conditions with various primary BMOs (0.5–2 mM as Mn) and exogenous Mn^{2+} (0–1.5 mM) as listed in Table 1. In the cases of sequestration experiments with exogenous Mn^{2+}, i.e., BMO_{1.0}/ExMn_{1.0} and BMO_{0.5}/ExMn_{1.5}, >98.6 ± 0.0% for V(V), >98.0 ± 0.1% for Mo(VI), and >94.6 ± 1.4% for W(VI) (Figure 4A–C) of exogenous Mn^{2+} were converted to solid phase Mn due to Mn(II) oxidation by the primary BMOs. In all cases, the resultant BMO phases comprised 80%–90% reducible (oxidized) Mn (Figure 4A–C). These results confirmed that there were no significant differences in the total concentration of solid Mn (acting as absorbents) at the termination of the sequestration experiments.
Normalized sequestration of V(V), Mo(VI), and W(VI) (mol/mol relative to reducible Mn), however, was considerably different among the experimental conditions with the orders of BMO_{0.5}/ExMn_{1.5} > BMO_{1.0}/ExMn_{1.0} > BMO_{2.0}/ExMn_{0.0} for all oxyanion species investigated. These results were consistent with the observation in Figure 3, demonstrating that enzymatic BMO formation readily enhances the sequestration of such oxyanion species. For BMO_{2.0}/ExMn_{0.0}, where no Mn(II) oxidation is concurrent with the sequestration process, maximum sequestration capacities deduced by the Langmuir isotherm fitting were 2.8 \((R^2 = 0.931)\), 0.6 \((R^2 = 0.870)\), and 2.2 \((R^2 = 0.826)\) mol\% (relative to reducible Mn) for V(V), Mo(VI), and W(VI), respectively (Figure 5A–C). These values are lower than those for heavy metal ions, such as Cd\(^{2+}\), Ni\(^{2+}\), and Zn\(^{2+}\) [29,32]. Thus “preformed” BMOs primarily have low capacities for sequestration for such anionic species. In contrast, maximum sequestrations for the case of BMO_{1.0}/ExMn_{1.0} were higher at 7.5 mol\% V(V) \((R^2 = 0.934)\), 2.7 mol\% Mo(VI) \((R^2 = 0.981)\), and 11.6 mol\% W(VI) \((R^2 = 0.976)\), increasing to 13.0 mol\% \((R^2 = 0.964)\), 8.7 mol\% \((R^2 = 0.985)\), and 20.6 mol\% \((R^2 = 0.948)\) for BMO_{0.5}/ExMn_{1.5} (Figure 5A–C). The latter values were 4.6, 14.5, and 9.4 times higher than those for BMO_{2.0}/ExMn_{0.0}. Enzymatic BMO formation should provide more sorption sites when these oxyanions coexist as absorbates. The normalized sequestration values at 8.8 mol\% for Mo(VI) and 28.8 mol\% for W(VI) observed in Section 3.1 were reasonable because all absorbent BMO phases were formed in the presence of Mo(VI) or W(VI) (Figure 2H,I).
Figure 5. Apparent Langmuir isotherms of (A) V(V), (B) Mo(VI), and (C) W(VI) sequestration under the experimental conditions of BMO$_{0.0}$/ExMn$_{0.0}$, BMO$_{1.0}$/ExMn$_{1.0}$, and BMO$_{0.5}$/ExMn$_{1.5}$, respectively (See Table 1). The amounts of oxyanions sequestered ($V_{\text{seq.}}$, $Mo_{\text{seq.}}$, and $W_{\text{seq.}}$) were normalized relative to reducible Mn. The error bars represent the standard deviation ($n=3$).

3.4. Mineralogical Characteristics of BMOs Formed with Coexisting V(V), Mo(VI), and W(VI)

Newly formed (primary) BMOs enzymatically deposited by A. strictum KR21-2 are referred to as a natural nanostructured and turbostratic variety of birnessite [27] of which the XRD peaks at ~7.3, 2.4, and 1.4 Å were assigned to (001), (11,20), and (31,02), respectively [39]. In all BMO$_{2.0}$/ExMn$_{0.0}$ cases, the resultant BMO after sequestration experiments showed a typical XRD pattern similar to that of the primary BMO (Figure 6A–D), suggesting that there were no apparent mineralogical alterations through the sequestration of V(V), Mo(VI), and W(VI) on the “preformed” BMO. For BMO$_{1.0}$/ExMn$_{1.0}$ with V(V), Mo(VI), and W(VI), the resultant BMOs displayed declines in XRD intensity arising from (001) basal reflection, while the corresponding XRD peaks disappeared almost entirely for BMO$_{0.5}$/ExMn$_{1.5}$ (Figure 6A–C). In contrast, the control experiments using SO$_4^{2-}$ (added as Na$_2$SO$_4$) did not affect the XRD patterns (Figure 6D). Thus, the more significant declines with increases in sequestration capacity suggested the disturbance of the ordering of the birnessite sheet stacking through the sorption of the oxyanions during enzymatic BMO formation. Generally, BMOs are negatively charged due to the high density of the Mn$^{IV}$ vacancy in their structure [27]. This structural vacancy is one of the main factors causing the high sorption capacity of BMOs for cationic species, such as heavy metal cations. From the mineralogical characteristics of BMOs, sequestration (sorption) of oxyanions could likely occur mainly at the edge sites through the surface complexation. For example, V(V) sorbes on a chemically synthesized birnessite by forming monodentate corning-sharing complexes [42] and by forming a bidentate mononuclear edge-sharing complex [43]. Kashi-
Wabara et al. [11], using wavelength dispersive X-ray absorption fine structure spectroscopy, demonstrated that Mn oxide is the main host phase for negatively charged WO$_4^{2-}$ through inner-sphere complex formation. Tanaka et al. [12] proposed the adsorption mechanism of Mo(VI) through the formation of disordered octahedral Mo(VI) species on Mn oxides. These studies [11,12] explain that such oxyanions are preferentially hosted by “negatively-charged” Mn oxide phases in nature. Such complexation and adsorption of the coexisting oxyanions on the birnessite sheet structure possibly resulted in the disturbance of the sheet stacking of the resultant BMOs. Further studies need to evaluate the atomic-level mechanisms of the sequestration processes during enzymatic BMO formation.

Figure 6. X-ray diffractions of the resultant BMOs after sequestration experiments for (A) V(V), (B) Mo(VI), and (C) W(VI) (~1 mM) under the experimental conditions of BMO$_{2.0}$/ExMn$_{0.0}$, BMO$_{1.0}$/ExMn$_{1.0}$, and BMO$_{0.5}$/ExMn$_{1.5}$, respectively (See Table 1). (D) Na$_2$SO$_4$ was used as the experimental control, where no apparent changes in the XRD patterns were observed. The error bars represent the standard deviation (n = 3). (See Table 1). The amounts of oxyanions sequestered (V$_{seq}$, Mo$_{seq}$, and W$_{seq}$) were normalized relative to reducible Mn. The error bars represent the standard deviation (n = 3).
4. Conclusions

The present study demonstrated that coexisting oxyanions, V(V), Mo(VI), and W(VI), readily affect the mineralogical feature of BMOs during ongoing formation and subsequently increase their sequestration capacity of the resultant BMOs. X-ray diffraction displayed a more significant decline of the peak arising from (001) basal reflection of turbostratic birnessite with increasing sequestration capacity. This suggested the disturbance of the ordering of the birnessite sheet stacking, possibly due to the complexation of oxyanions on the edge sites of the sheet structure of BMOs. These processes are mediated by the Mn(II)-oxidizing activity, and consequently, the enzymatic active BMO is considered a valuable tool to recover (remove) oxyanionic elements from the contaminated water. Additionally, this study strongly infers biological (fungal and bacterial) contributions to the accumulation of V(V), Mo(VI), and W(VI) in Mn oxide phases through enzymatic formation in natural environments.

Author Contributions: Conceptualization, Y.T., T.W., T.S. and N.M.; methodology, Y.T., T.W., T.S. and N.M.; validation, Y.T., K.U. and N.M.; investigation, Y.T., T.W. and N.M.; data curation, Y.T., K.U. and N.M.; writing—original draft preparation, Y.T., T.W. and T.S.; writing—review and editing, K.U. and N.M.; visualization, Y.T., T.W. and T.S.; supervision, Y.T.; project administration, Y.T. and N.M.; funding acquisition, Y.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Japan Society for the Promotion of Science, JSPS KAKENHI, grant no. 20K12222 (Y.T.).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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