Phosphorus recovery from artificial wastewater by microbial fuel cell and its effect on power generation

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Abstract

The effects of ammonium (NH\textsubscript{4}) and magnesium (Mg) on the precipitation of phosphorus in artificial wastewater by an air-cathode single-chamber microbial fuel cell were investigated. When both NH\textsubscript{4} and Mg were added to the wastewater, phosphorus was precipitated as struvite. Almost no precipitation occurred with the addition of only NH\textsubscript{4}, while phosphorus was precipitated as cattiite with the addition of only Mg. However, the amount of precipitate was less than that observed in experiments in which NH\textsubscript{4} was also added. As the amounts of NH\textsubscript{4} and Mg were increased, more precipitate was observed. Precipitated phosphorus on the cathode was recovered by dissolution in Milli-Q water and MES buffers. It was discovered that the formation of a precipitate reduced the performance of the cathode. Dissolution treatment caused the performance of the cathodes to increase to their initial level.
Keywords
Microbial fuel cell; phosphorus recovery; struvite; cattiite; cathode performance; cathode regeneration

1. INTRODUCTION

The microbial fuel cell (MFC) is a technology that converts energy in an organic substrate in wastewater into electrical energy with the aid of electrogenic microorganisms (Kim et al., 1999, 2002; Bond and Lovley, 2003). Because wastewater throughout the world contains a substantial amount of energy, its utilization as a power source has significant potential in helping attain a sustainable society. Therefore, MFCs are regarded as a promising technology.

Phosphorus is also a valuable resource contained in wastewater because it is an essential nutrient for plant growth (Zhang et al., 2003) and is used as fertilizer. Although its depletion would result in a reduction in food production, phosphorus as a natural resource is undergoing a crisis of short supply. It has been suggested that the remaining accessible reserves of clean phosphate rock will be depleted within 50 years (Gilbert, 2009). Therefore, the recovery of phosphorus from wastewater has become a pertinent issue worldwide.

In a previous study, it was found that a precipitate containing a high concentration of phosphorus was formed on the cathode of an MFC during power generation (Hirooka et al., 2011; Ichihashi et al., 2012; Ichihashi and Hirooka, 2012). Because the main component of the precipitate was struvite, the following hypothesis regarding the mechanism of precipitation was formulated (Ichihashi and Hirooka, 2012): through the oxygen reduction reaction, the pH near the cathode locally increases (Zhao et al., 2006), and the solubility of phosphate, ammonium, and magnesium reaches
oversaturation, causing precipitation. If this hypothesis is true, then the phosphorus in wastewater can be recovered with the addition of ammonium (NH\textsubscript{4}) and magnesium (Mg) during MFC treatment. Moreover, as this method does not require a pH adjustment, it has a significant advantage over conventional struvite crystallization methods because the addition of alkali chemicals required to adjust the pH is expensive (Jaffer et al., 2002).

Therefore, to test the hypothesis, NH\textsubscript{4} and Mg was added to artificial wastewater containing phosphorus and treated the water with an MFC. Subsequently, the concentrations of NH\textsubscript{4} and Mg were altered, and the effect of this change was examined. Cases in which either NH\textsubscript{4} or Mg alone was added to the wastewater were tested as well. Additionally, the precipitate from the cathode needed to be collected to recover the phosphorus in it; thus, a precipitate dissolution treatment was performed to confirm the recovery rate.

Moreover, it was considered that the formation of a precipitate might reduce the performance of the cathode. There are some reports that indicate that precipitation degrades cathode performance (Chung et al., 2011; Jiang et al., 2011; Saito et al., 2011; Zhuang et al., 2012). Therefore, the cathode performance before and after the precipitation tests was measured using linear sweep voltammetry (LSV) tests. Furthermore, LSV tests was used to measure the cathode performance before and after the removal of the precipitate by the dissolution treatment described above.

2. MATERIALS AND METHODS

2.1. MFC configuration and acclimation

Air-cathode single-chamber MFCs (Figure 1) were used in this study, with 70 mL as the working volume of the anode chamber. The cathode was prepared using a coating of 0.5 mg/cm\textsuperscript{2} of a Pt/C catalyst on wet-proofed porous carbon paper (Toray Carbon Paper TGP-H-120, Toray Industries, Inc., Japan), as previously described by Cheng et al. (2006). The effective projected area of the
cathode was 47 cm$^2$ (7.7 cm diameter). Carbon felt discs (LFP-210, Osaka Gas Chemicals Co., Ltd., Japan) with a diameter of 7.7 cm (47 cm$^2$ projected surface area) and thickness of 1 cm were used as the anode electrodes in the anode chambers. A graphite rod was laid on the anode to fix the anode’s position, and one end of the rod was connected to an external circuit. A small piece of a nonwoven polyester cloth separator (2 cm × 3.5 cm; 0.5 cm thickness) was placed against both the carbon rod and the cathode to prevent contact. The MFC was connected to a glass bottle with a volume of 300 mL, and the internal liquid was recirculated between the MFC and the bottle, as previously described by Borole et al. (2009), at a flow rate of 20 mL/min. Every 5-10 days, the bottle was replaced with a new bottle filled with artificial wastewater without substrate, while a syringe pump continuously added the substrate. There was an external resistance of 10 Ω between the anode and the cathode.

The MFCs were inoculated with part of a biofilm formed from a previously operated MFC and acclimated for 108 days using artificial wastewater composed of a substrate (sodium acetate), buffered medium (phosphate buffer or HEPES buffer), trace minerals (final concentration of nitrilotriacetic acid NTA: 19 mg/L, MgSO$_4$: 38 mg/L, MnSO$_4$·H$_2$O: 6.3 mg/L, NaCl: 13 mg/L, FeSO$_4$·7H$_2$O: 1.3 mg/L, CaCl$_2$·2H$_2$O: 1.3 mg/L, CoCl$_2$·6H$_2$O: 1.3 mg/L, ZnCl$_2$: 1.6 mg/L, CuSO$_4$·5H$_2$O: 0.13 mg/L, AlK(SO$_4$)$_2$·12H$_2$O: 0.13 mg/L, H$_3$BO$_3$: 0.13 mg/L, Na$_2$MoO$_4$: 0.31 mg/L, NiCl$_2$·6H$_2$O: 0.30 mg/L, Na$_2$WO$_4$·2H$_2$O: 0.31 mg/L), and vitamins (final concentration of biotin: 2.5 mg/L, folic acid: 2.5 mg/L, pyridoxine HCl: 13 mg/L, riboflavin: 6.3 mg/L, thiamin: 6.3 mg/L, nicotinic acid: 6.3 mg/L, pantothenic acid: 6.3 mg/L, B-12: 0.13 mg/L, p-aminobenzoic acid: 6.3 mg/L, thioctic acid: 6.3 mg/L). The concentration of sodium acetate added was initially 0.81 mmol/L-day and was gradually increased over a period of 21 days as the MFCs’ performance increased; this concentration was finally fixed at 8.1 mmol/L-day during the last 75 days of acclimation. Phosphate-buffered medium (NaH$_2$PO$_4$: 36 mM, Na$_2$HPO$_4$: 64 mM, NH$_4$Cl: 12 mM,
KCl: 3.5 mM, pH 6.8) was used during the first 71 days of acclimation, and HEPES-buffered medium (C$_8$H$_{18}$N$_2$O$_4$S[HEPES]: 100 mM, NaCl: 110 mM, adjusted to pH 6.8 with NaOH) was used during the final 37 days to exclude phosphorus from the internal liquid. The electrical conductivities of the phosphate-buffered medium and the HEPES-buffered medium were 11.2 mS/cm and 11.3 mS/cm, respectively.

2.2. Precipitation tests

A series of precipitation tests were conducted using the acclimated MFCs. In all four tests, the MFCs were operated with artificial wastewater composed of sodium acetate (at 8.1 mmol/L-day), HEPES-buffered medium, trace minerals, and vitamins at room temperature. Two to three days before each test, the air cathodes, the separators, and the bottles were replaced with new ones. After each test, the used cathodes and separators were replaced with those used for maintenance. The inside of each MFC was flushed with HEPES-buffered medium three times, and the MFCs were operated for 4-5 days until the next precipitation test.

In Test 1, 3.75 mL of phosphate solution (Na$_2$HPO$_4$: 360 mM, Na$_2$HPO$_4$: 640 mM, pH 6.8) was added to three MFC bottles. After the addition, the theoretical P concentration in the internal liquid was 10 mM. A “NH$_4$ + Mg solution” containing both NH$_4$ and Mg, with theoretical concentrations of 15 mM each, was added to only one MFC. To the other two MFCs, a “NH$_4$-only solution” and “Mg-only solution” were added, with the theoretical concentrations of NH$_4$ and Mg set at 15 mM each. Thirty minutes before the start of the experiment, phosphate solution was added to the MFC bottles. At 0 hr, the internal liquid was sampled from each MFC, and the “NH$_4$ + Mg solution”, “NH$_4$-only solution”, and “Mg-only solution” were added to each MFC using a syringe pump over 0.5 hr. At 1 hr, 25 hr, 49 hr, 73 hr, 97 hr, and 121 hr, the internal liquid was sampled from the three MFCs, and at 121 hr, Test 1 was completed.

In Test 2, the same concentration of phosphate solution used in Test 1 (pH: 6.8, theoretical P
concentration: 10 mM) and three different concentrations of the “NH₄ + Mg solution” (theoretical concentrations: 5 mM, 10 mM, and 15 mM of NH₄Cl and MgSO₄, each) were added to three MFC bottles. Thirty minutes before the start of the experiment, phosphate solution was added to the MFC bottles. At 0 hr, the internal liquid was sampled from each MFC, and the appropriate concentration of “NH₄ + Mg solution” was added to each bottle using a syringe pump over 0.5 hr. At 1 hr, 25 hr, 49 hr, 73 hr, 97 hr, and 121 hr, the internal liquid was sampled from the three MFCs, and at 121 hr, Test 2 was completed.

In Test 3, phosphate solution (pH: 6.8) was added to two MFC bottles (theoretical concentration: 33 mM), and “the NH₄ + Mg solution” was continuously added to only one MFC bottle. One hour before the start of the experiment, phosphate solution was added to the two MFC bottles. Starting at 0 hr, 7.5 mL of “NH₄ + Mg solution” (NH₄Cl: 1000 mM, MgSO₄: 1000 mM) was continuously added to one MFC over 96 hours using a syringe pump at a rate of 1.875 mL/day. To the other MFC, Milli-Q water was added under the same conditions. At 0 hr, followed by 24 hr, 48 hr, 72 hr, 96 hr, and 100 hr, the internal liquid was sampled from the two MFCs, and at 100 hr, Test 3 was completed.

2.3 Precipitation test without MFC operation
To confirm that precipitation does not occur without an MFC under the same concentrations of phosphate, NH₄, and Mg used in Precipitation Tests 1, 2, and 3, control tests were carried out. Three beakers filled with 100 mL of HEPES-buffered phosphate solution (C₈H₁₈N₂O₄S[HEPES]: 100 mM, NaCl: 110 mM, NaH₂PO₄: 10 mM, adjusted to pH 6.93 with NaOH) were prepared, and three different concentrations of “NH₄ + Mg solution” (theoretical concentrations: 5 mM, 10 mM, and 15 mM of NH₄Cl and MgSO₄, each) were added while the solution in the beakers was mixed. Then, NaOH solution (NaOH: 6 M) was gradually added under pH monitoring until precipitate formation was observed.
Moreover, to confirm that a precipitate does not form on the surface of the cathode without MFC operation, a piece of air cathode (1 cm x 2 cm) was soaked in 100 mL of HEPES-buffered phosphate solution with “NH₄ + Mg solution” (C₈H₁₈N₂O₄S[HEPES]: 100 mM, NaCl: 110 mM, NaH₂PO₄: 10 mM, NH₄Cl: 10 mM, MgSO₄: 10 mM, trace minerals and vitamins [concentrations described above], adjusted to pH 6.93 with NaOH) for 5 days.

2.4. Measurement of cathode performance and dissolution treatment

Before Precipitation Test 3, the performance of each cathode was evaluated by linear sweep voltammetry (LSV) analysis under the conditions described below. After Precipitation Test 3, the used cathodes were soaked in a tray of Milli-Q water and then rinsed by stirring gently for 10-20 seconds. The rinsed cathodes were dried at room temperature and subjected once more to LSV analysis.

After the second LSV analysis, the precipitate on the cathodes was removed by immersion in a dissolution solution. After an initial immersion for 30 hours, the dissolution solution was replaced five times. The first, second, and third solutions were 500 mL of Milli-Q water (the theoretical pH of Milli-Q water is 7; however, the pH was not actually measured because it is difficult to measure the pH of samples with low conductivity), and the fourth, fifth, and sixth solutions were 500 mL of MES buffer (C₆H₁₃NO₄S[MES]: 10 mM, adjusted to pH 5.5 with NaOH). The total immersion time in Milli-Q water was 8 days, and the total immersion time in MES buffer was 4 days. The treated cathodes were rinsed and dried and subjected to a third LSV analysis.

LSV was performed to assess electrochemical cathode performance. A single-chambered cell, which had the same structure as the MFCs used in the precipitation tests, was filled with HEPES-buffered medium (C₈H₁₈N₂O₄S[HEPES]: 100 mM, NaCl: 110 mM, adjusted to pH 6.8 with NaOH) and equipped with the cathode as the working electrode, a carbon felt disc with a diameter...
of 7.7 cm (1 cm thickness) as the counter electrode, and an Ag/AgCl electrode as the reference electrode. LSV curves were obtained using a potentiostat (HZ-5000, Hokuto Denko Corporation, Japan) from the measured open-circuit voltage (OVC) to −0.2 V (vs. Ag/AgCl) at a scan rate of 1 mV/s.

2.5. Analytical methods

The electric potential difference (voltage: U) across an external resistor in the MFC circuit was monitored using a data logger (midi LOGGER GL200A, Graphtec Corporation, Japan). The current (I = U/R) was calculated using the values of U and the external resistance (R), and the current density was normalized to the projected surface area of the cathode. The coulombic efficiency was calculated by integrating the measured current relative to the theoretical maximum current, assuming that the chemical oxygen demand (COD) of the substrate was completely depleted.

The internal liquid in the MFCs was filtered through a 0.45 μm membrane filter. The used cathodes were rinsed and dried by the same process mentioned above, and the dried precipitate on the cathode was scraped off with a plastic pipette tip to prevent contamination of the catalyst layer. The concentration of ammonium (NH₄) in the filtered internal liquid was measured utilizing the Hach Laboratory Method 10023 (DR/870, Hach, USA). The filtered liquid and the precipitate were digested with nitric acid by heating at 115 °C for 60 min. The digested samples were analyzed for phosphorus (P) and Mg using ICP-AES (ULTIMA 2, Horiba, Japan).

The dried precipitate was milled in an agate mortar and was characterized over a period of 30 minutes from 10° to 70° by X-ray diffraction (XRD) using an ULTIMA IV diffractometer (Rigaku, Japan) operated at 40 kV and 30 mA. Qualitative analysis was performed using PDXL software (Rigaku, Japan).
3. RESULTS AND DISCUSSION

3.1. Effects of the presence of NH$_4$ and/or Mg (Precipitation Test 1)

The concentrations of P and Mg in the internal liquid of the MFCs during Test 1 are shown in Figure 2. In the MFC that was supplied 15 mM each of NH$_4$ and Mg (MFC[NH$_4$ and Mg]), the P concentration promptly decreased from 11 mM to 10 mM during the first hour after Test 1 started. Over the following 4 days, it gradually decreased to 4.8 mM and remained there until the end of the test (day 5). In contrast, in the MFC that was supplied 15 mM of Mg (MFC[Mg]), the P concentration decreased to approximately 9.3 mM during the first hour; however, after that, it decreased only slightly. In the MFC that was supplied 15 mM of NH$_4$ (MFC[NH$_4$]), the P concentration showed a similar trend to that in MFC[Mg]; it did not significantly decrease after the first hour, and at the end of the test, it was approximately 10 mM.

The Mg concentration in MFC[NH$_4$ and Mg] started at 14 mM and decreased gradually with a decrease in the P concentration; it dropped to 7.6 mM at the end of the test. In MFC[Mg], although the P concentrations did not decrease as much as in MFC[NH$_4$ and Mg], the Mg concentrations started at 13 mM and gradually decreased until the end to 9.0 mM. After Test 1, the pH levels of the internal liquids in MFC[NH$_4$ and Mg], MFC[Mg], and MFC[NH$_4$] were 6.93, 6.68, and 6.90, respectively.

Precipitate was observed only on the surface of the liquid side of the cathodes in MFC[NH$_4$ and Mg]. Moreover, a small quantity of precipitate was observed on the cathode in MFC[Mg]; however, its amount and physical appearance differed from that of MFC[NH$_4$ and Mg] (Figure S1). Almost no precipitate was observed on the cathode in MFC[NH$_4$]. The X-ray powder diffraction spectra of the above precipitates were recorded (Figure 3). The spectrum pattern from MFC[NH$_4$ and Mg] matched the reference pattern of synthesized struvite (NH$_4$MgPO$_4·$6H$_2$O), and by qualitative
analysis, only struvite was detected. On the other hand, the pattern from MFC[Mg] matched the reference pattern of synthesized cattiite (Mg₃(PO₄)₂·22H₂O). The quantity of precipitate in MFC[NH₄] was too small to analyze, but it was most likely struvite because a small amount of Mg was contained in the mineral solution.

With the addition of both NH₄ and Mg, 55 % of P was removed mainly as struvite precipitate from the wastewater. However, with the addition of only Mg, 19 % of P was removed mainly as cattiite precipitate. Moreover, the fact that precipitation occurred only on the cathode surface is consistent with previous results reported for the treatment of swine wastewater (Ichihashi and Hirooka, 2012). This finding supports the hypothesis that precipitation occurred due to a local increase in pH.

Cusick and Logan (2012) reported that phosphate was recovered as struvite from synthetic wastewater by a microbial electrolysis cell. They also stated that phosphate removal likely resulted from a local pH increase near the cathode.

3.2. Effects of the concentrations of NH₄ and Mg (Precipitation Test 2, Test 3)

The concentrations of P and Mg in the internal liquid of the MFCs during Test 2 are shown in Figure 4. In the MFC that was supplied 15 mM of NH₄ and Mg (MFC[15 mM]), the P concentration promptly decreased from 11 mM to 9.0 mM during the first hour after the start of Test 2. Over the following 2 days, it gradually decreased below 6.0 mM and remained stable until the end of the test.

In the MFC that was supplied 10 mM of NH₄ and Mg (MFC[10 mM]), the P concentration decreased to approximately 7.0 mM during the first hour; however, after that, there was no further decrease. In the MFC that was supplied 5 mM of NH₄ and Mg (MFC[5 mM]), a similar trend to that of MFC[10 mM] was observed; the P concentration did not significantly decrease after the first hour, and at the end of the test, it was approximately 8.9 mM.
The Mg concentration in MFC[15 mM] started at 14 mM, and it decreased gradually until the end of the test to 5.5 mM, although the P concentration no longer decreased after day 2. In MFC[10 mM], the Mg concentration started at 6.3 mM and decreased gradually until the end of the test to 4.6 mM. Additionally, the Mg concentration in MFC[5 mM] remained nearly constant after the first hour until the end of the test, when it was approximately 2.7 mM. After Test 2, the pH levels of the internal liquids in MFC[5 mM], MFC[10 mM], and MFC[15 mM] were 6.76, 6.64, and 6.72, respectively. The residual concentrations of NH$_4$ were 2.0 mM (MFC[5 mM]), 5.4 mM (MFC[10 mM]), and 9.1 mM (MFC[15 mM]).

As in MFC[NH$_4$ and Mg] in Test 1, precipitate was observed only on the surface of the liquid side of the cathodes in all MFCs. There was a tendency for the amount of precipitate to increase as the amount of NH$_4$ and Mg added increased (Figure S2). The XRD spectrum of the precipitate in MFC[15 mM] was recorded, and it was confirmed that the main component of the precipitate was struvite. Based on the results of elemental analysis by ICP-AES, 16 % (w/w) of P and 19 % (w/w) of Mg were contained in the precipitate. The molar ratio of Mg:P in the precipitate was 1.6:1, which differed from the theoretical molar ratio of P:Mg (1:1), assuming that the precipitate was composed of 100 % struvite. This result suggests that the precipitate was not composed of pure struvite and contained other magnesium compounds.

In short, with increasing amounts of NH$_4$ and Mg, a higher amount of P was removed from the wastewater as a result of enhanced struvite precipitation.

The concentrations of P and Mg in the internal liquid of the MFCs during Test 3 are shown in Figure 5. In the MFC that was supplied NH$_4$ and Mg (MFC[Add]), the P concentration continuously decreased from 32 mM to 18 mM over the duration of Test 3. The rate of decrease over the following 2 days was greater than that over the initial 2 days. The concentration of Mg increased proportionally until the second day to 7.6 mM. After day 2, it gradually decreased, and the final
concentration on day 5 was 6.0 mM. On the other hand, in the MFC that was supplied Milli-Q water (MFC[Blank]), the decrease in the P concentration was not greater than the theoretical decrease due to the dilution effect (0.8%/day) over 4 days of operation.

After the test, struvite precipitate was observed only on the surface of the liquid side of the cathodes in MFC[Add]. No precipitate was observed in MFC[Blank].

3.3. Confirmation of the absence of precipitation without MFC operation

When 5, 10, or 15 mM of “NH₄ + Mg solution” was added to buffer solutions containing 10 mM of phosphate, no precipitate was observed. Subsequently, NaOH solution was gradually added, and precipitation was then observed at pH 8.62 in the solution with 5 mM of “NH₄ + Mg solution”, at pH 7.63 in the solution with 10 mM “NH₄ + Mg solution”, and at pH 7.35 in the solution with 15 mM “NH₄ + Mg solution”. The fact that the pH values of the internal liquid in the MFCs after Precipitation Tests 1 and 2 ranged from 6.63 to 6.93 indicates that the pH in the bulk liquid during the tests was not high enough to form a precipitate.

Moreover, when a piece of air cathode was soaked for 5 days in a buffer solution of pH 6.93 with 10 mM each of phosphate, NH₄, and Mg, no precipitate was observed. On the other hand, in Precipitation Test 2, precipitate was observed on the cathode of MFC[10 mM], which was operated with the same concentrations of phosphate, NH₄, and Mg. These results demonstrate that MFC operation was necessary for precipitation on the cathodes.

In short, it was confirmed that precipitation does not occur anywhere in the bulk liquid of the MFCs but occurs only near the cathode surface during MFC operation.

3.4. Current production in the MFCs during precipitation tests

In Test 1, before adding the “NH₄ + Mg solution”, “NH₄-only solution”, or “Mg-only solution”, the
three MFCs showed more than 4.3 A/m² of current production (Figure S3). After the addition, the current production in MFC[NH₄+Mg] decreased compared to that in MFC[NH₄] and MFC[Mg]. Moreover, the current fluctuated more than that in the remaining two MFCs, especially during the initial two days. In Test 2, a similar trend was observed for MFC[15 mM]; a fluctuation in the current production was observed and was lower than that in the remaining two MFCs (MFC[10 mM], MFC[5 mM]). In Test 3, the current production in MFC[Add] was also less than that in MFC[Blank], and the fluctuation was larger during the latter part of the test. Immediately before all the tests, the coulombic efficiency of all the MFCs was greater than 73 %. In conclusion, it is clear that the current production in the MFCs with greater amounts of precipitate was lower compared to that in the MFCs with little or no precipitate. This result may be due to the fact the mass transfer near the cathode was impeded by the precipitate.

3.5. The effects of NH₄ and Mg on the formation of precipitate containing phosphorus

In Test 1, when both NH₄ and Mg were added to the wastewater containing phosphorus, struvite was precipitated. Almost no precipitate was observed when only NH₄ was added; however, cattiite was precipitated instead of struvite when only Mg was added, as mentioned above. Cattiite is a type of magnesium phosphate, and it is natural that magnesium phosphate precipitates under the conditions mentioned above with PO₄ and Mg and without NH₄. It is reported that under such circumstances, the predominant precipitate in the solution, for example, cattiite or another magnesium phosphate (for example, newberyite [MgHPO₄·3H₂O], magnesium phosphate pentahydrate [Mg₃(PO₄)₂·5H₂O], or brucite [Mg(OH)₂]), varies according to conditions such as temperature, pH, and NH₄ or Mg concentrations (Tamimi et al., 2011). Therefore, for the MFCs also, phosphate compounds other than struvite or cattiite might precipitate on the cathode depending on the concentrations of NH₄ or Mg and the operating conditions. Tamimi et al. (2011) reported that
when a solution containing 6.7 mM of Mg and 10 mM of phosphate was mixed for 24 hours at 21 °C under various pH conditions, cattiite was not precipitated at pH 8 but instead at pH 10.

Because the Mg and PO$_4$ concentrations in Test 1 were at nearly the same, the local pH near the cathode in the MFCs might be increased to values greater than 8. Regarding the local pH increase near the cathode, it is referred to by Zhao et al. (2006) and Yan et al. (2012). Yan et al. (2012) carried out a polarization experiment using MFCs with a nitrifying biofilm enriched at the air cathode, and after the experiment, ammonia removal in the MFCs never recovered to its previous level. The researchers suggested that the larger current generated when small external resistors were connected to the MFCs may promote a larger pH increase within the cathode biofilm, which might cause the death of some nitrifiers on the cathode.

Regarding the effect of concentration, in Test 2 (initial phosphate concentration 10 mM), more precipitation was observed as greater amounts of NH$_4$ and Mg were added. These results indicate that higher concentrations of NH$_4$ and Mg are required to cause struvite precipitation in wastewater containing a lower concentration of phosphate. This behavior may arise because the precipitation of struvite on the cathode is based on the theory of solubility.

Struvite precipitation occurs when the product of the concentrations of magnesium, ammonium, and phosphate ions exceeds the solubility product (often denoted as $K_{sp}$, the value has been reported to be 3.98 x 10$^{-10}$ - 7.08 x 10$^{-14}$ [Doyle and Parsons, 2002]) of struvite. However, the product calculated from the measured concentrations of ions in solution is generally much higher than the solubility product because the actual solubility of struvite is affected by chemical speciation, ionic strength, ionic activity, and supersaturation. For example, in the study of Stratful et al. (2001), the residual concentrations of NH$_4$, Mg, and PO$_4$ ranged from 7 mM to 13 mM, from 0.5 mM to 7 mM, and from 6 mM to 23 mM, respectively, in the solutions after struvite crystallization batch tests (pH was controlled between 7.5 and 10). The product of the ion concentrations calculated from the
above concentrations ranged from $2.4 \times 10^{-8}$ to $1.9 \times 10^{-6}$. In this study, the products of the residual concentrations of NH$_4$, Mg, and P after Precipitation Test 2 were calculated to be $4.8 \times 10^{-8}$ (MFC[5 mM]), $1.8 \times 10^{-7}$ (MFC[10 mM]), and $2.9 \times 10^{-7}$ (MFC[15 mM]). These values are consistent with the results of Stratful et al (2001). Moreover, there was a tendency for the product of the residual ion concentrations to increase as the amount of precipitate on the cathode increased; in other words, the residual concentrations of ions were higher. This behavior may have arisen because when the cathode surface was covered with precipitate, the diffusion of ions to the region of higher pH near the cathode was blocked.

The concentrations of NH$_4$ and Mg required for precipitation in wastewater with a low phosphorus concentration, such as municipal wastewater (0.03 – 0.3 mM [Zhang et al., 2011]), should be investigated while considering cost efficiency in a future study to clarify the practical limits of phosphorus removal.

3.6. Recovery of phosphorus from the cathode

After Precipitation Test 3, the cathodes from MFC[Add] and MFC[Blank] were treated by immersion in dissolution solution. The precipitate crystal on the cathode of MFC[Add] was dissolved in the solution and finally disappeared. After dissolution treatment, the pH values of the used Milli-Q water were 9.57 (first dissolution), 7.63 (second), and 9.02 (third), while on the other hand, those of the used MES buffer were 6.30 (fourth), 6.13 (fifth), and 5.86 (sixth). The concentration of phosphorus in the solution was measured, and it was revealed that the amount of phosphorus dissolved in the MES buffer was approximately 3.5 times larger than that in the Milli-Q water. The reason that the precipitate dissolved to a greater extent in the MES buffer is believed to be that the solubility of struvite increased in the lower-pH solution (Doyle and Parsons, 2002; Marti et al., 2008). It is estimated that 27% of the phosphorus added to MFC[Add] was recovered in the
dissolution solution. Less than 0.01 % remained on the cathode. On the other hand, the amount of phosphorus removed from the internal liquid was estimated to be 40 % of the added phosphorus. Therefore, 31 % was lost during the LSV analyses and rinsing with Milli-Q water in the dissolution procedure.

The molar ratio of Mg:P in the dissolution solution (Test 3) was 1.4:1, which is different from the theoretical molar ratio of struvite (Mg:P =1:1) and different from the molar ratio in the precipitate analyzed in Test 2 (Mg:P =1.6:1). Because these two tests were performed under varying precipitation conditions, these results cannot be simply compared. However, regardless, the fact that the Mg:P ratios in both Test 3 and Test 2 were not 1:1 indicates that the precipitate was not composed of pure struvite and contained other magnesium compounds. Such compounds were not detected in the precipitate by XRD analysis. This might be because they were not in crystal form but were rather amorphous, or they might be inhomogeneous and the sample for the ICP-AES elemental analysis may have incidentally contained more magnesium compounds.

3.7. Deterioration and regeneration of the cathode

Figure 6 shows the results of the linear sweep voltammetry analysis of the cathodes. After Precipitation Test 3, the performance of the cathodes in both MFC[Add] and MFC[Blank] was reduced compared with that before the test. In particular, the deterioration of the cathode in MFC[Add] was notably greater than that in MFC[Blank]. After dissolution treatment, the performance of both cathodes recovered to their initial level and both cathodes showed almost the same performance.

From the results discussed, the decrease in cathode performance was presumably because the precipitate to some extent blocks mass transfer near the cathode. This is consistent with the finding that the current generated by the MFCs with precipitate was lower than that of the MFCs without
precipitate. This result indicates that, for the recovery of phosphorus by MFC in conjunction with good power generation, it is important to remove the precipitate from the cathode.

Moreover, the performance of the cathode in MFC[Blank] (with no significant precipitate) also decreased somewhat after operation and increased again after dissolution treatment. This likely indicates that some chemical compounds, which were in quantities too low to detect but sufficiently high for the prevention of mass transfer, were precipitated in the cathode and resulted in the drop in cathode performance. Dissolution treatment most likely removed the chemical compounds, resulting in the increase in performance.

Although the decrease in cathode performance occurred after only 4 days in this study, previous studies have reported decreases following long operation periods (Zhang et al., 2009; Chung et al., 2011; Jiang et al., 2011; Kiely et al., 2011; Saito et al., 2011; Zhuang et al., 2012). Saito et al. (2011) hypothesized that the deterioration was due to the development of a biofilm on the cathode and tried to remove it from a cathode that had been used for 40 days. After the removal of the biofilm, the cathode performance increased to some extent; however, it was still lower than that originally obtained during the initial stage (day 2) of operation with a fresh cathode. Kiely et al. (2011) also attempted to use a similar approach; however, the performance of the cathode, when the biofilm was removed, was still lower than that of the fresh replacement cathode. Kiely et al. concluded that biofilm development was not the primary reason for the deterioration of the cathodes. Zhuang et al. (2012) suggested that the formation of alkali salt in the cathodes could cover the catalyst and make oxygen contact with the catalyst difficult. To remove the alkali salt, they tried rinsing a cathode that had been used for 60 days with water and confirmed that the performance returned to the level observed on day 20.

In this study, the performance of both cathodes increased with dissolution treatment, presumably because the precipitate (struvite or other chemical compounds) in or on the cathodes was removed. This finding indicates that dissolution treatment can restore the cathode performance. Moreover, the
fact that the performance of the cathodes increased to their initial level may indicate that dissolution
treatment can remove chemical compounds that are initially contained in new cathodes and that
inhibit cathode reaction.

4. CONCLUSION

When both ammonium and magnesium were added, the phosphorus in artificial wastewater was
removed as struvite precipitate on the cathode of a single-chamber microbial fuel cell. When only
Mg was added, phosphorus was removed as cattiite; however, the extent of removal was not as
great as in the case of struvite. Larger amounts of precipitate were observed as greater amounts of
NH₄ and Mg were added. Precipitated phosphorus caused a decrease in cathode performance, and
the removal of precipitate through dissolution treatment with Milli-Q water and MES buffer
increased the performance of the cathodes to their initial levels.

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Figure titles

Figure 1. Configuration of the air-cathode single-chamber MFC

Figure 2. Concentrations of (a) P and (b) Mg in the internal liquid of the MFCs during Precipitation Test 1

Figure 3. XRD patterns of precipitates on the cathode in (a) MFC[NH₄+Mg], (b) struvite standard, (c) precipitates on the cathode in MFC[Mg], and (d) cattiite standard

Figure 4. Concentrations of (a) P and (b) Mg in the internal liquid of the MFCs during Precipitation Test 2

Figure 5. Concentrations of (a) P and (b) Mg in the internal liquid of the MFCs during Precipitation Test 3

Figure 6. LSV results regarding cathode performance: (a) comparison before and after Precipitation Test 3, (b) comparison after Precipitation Test 3 and after dissolution treatment, and (c) comparison before Precipitation Test 3 and after dissolution treatment

Figure S1. Photos of the cathodes in MFC[NH₄+Mg], MFC[NH₄], and MFC[Mg]

Figure S2. Photos of the cathodes in MFC[5 mM], MFC[10 mM], and MFC[15 mM]

Figure S3. Current production in MFCs during Precipitation Test 1
FOOTNOTE

1. These authors contributed equally to the research.
Figure 1

- Wastewater bottle (300ml)
- Pump
- Biocatalytic Anode (carbon felt 47cm²)
- Air Cathode (carbon paper 47cm²)
- Voltmeter
- 20 mL/min flow rate
- Substrate
- Anode chamber (70ml)
Figure 3

(a) Precipitate on the cathode in MFC [NH₄+Mg]

(b) Synthetic Struvite (Standard)

(c) Precipitate on the cathode in MFC [Mg]

(d) Synthetic Cattiite (Standard)

Intensity (cps)

Intensity (%)

Position [2theta]
Figure 6
Artificial wastewater containing phosphorus was treated by single chamber MFCs.

With NH$_4$ and Mg, struvite precipitated on the cathode, with Mg alone, cattiite.

As the amounts of NH$_4$ and Mg were increased, the more precipitate was observed.

Formation of precipitate caused a decrease in performance of the cathode.

By the removal of precipitate, cathode performance recovered to the initial level.