Isolation and Characterization of Coagulant Extracted from *Moringa oleifera* Seed by Salt Solution

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ABSTRACT

It is known that *M. oleifera* contains a natural coagulant in the seeds.   In our previous research, the method using salt water to extract the active coagulation component from *M. oleifera* seeds was developed and compared with the conventional method using water.   In this research, the active coagulation component was purified from a NaCl solution crude extract of *Moringa oleifera* seeds.   The active component was isolated and purified from the crude extract through a sequence of steps that included salting-out by dialysis, removal of lipids and carbohydrates by homogenization with acetone, and anion exchange.   Specific coagulation activity of the active material increased up to 34 times more than the crude extract after the ion exchange.   The active component was not the same as that of water extract.   The molecular weight was about 3000 Da.   The Lowry method and the phenol-sulfuric acid method indicated that the active component was neither protein nor polysaccharide.   The optimum pH of the purified active component for coagulation of turbidity was pH 8 and above.   Different from the conventional water extracts, the active component can be used for waters with low turbidity without increase in the dissolved organic carbon concentration.

KEYWORDS

Coagulation, purification, isolation, characterization, *Moringa oleifera*; natural coagulant

INTRODUCTION

Many coagulants are widely used in conventional water treatment processes for tap water production. These coagulants can be classified into inorganic coagulant, synthetic organic polymer and naturally occurring coagulant.   These coagulants are used for various purposes depending on their chemical characteristics.   An inorganic polymer "PAC" (polyaluminum chloride) and inorganic salt "alum" (aluminum sulfate) are the most widely used coagulants in water treatment (Van Benchosten and Edzwald, 1990; Boisvert *et al.,* 1997; Najm *et al.,* 1998) .   Recently, synthetic organic polymers became widely used for water treatment (Robinson, 1979; Teh and Ghosh, 1981; Lee *et al.,* 1998) .

However, there is a fear that aluminum (the major component of PAC and alum) may induce Alzheimer's disease (Crapper *et al.,* 1973; Miller *et al.,* 1988; Martyn *et al.,* 1989) and strong carcinogenic properties (Dearfield *et al.,* 1964; Mccollister *et al.,* 1964; Mallevialle *et al.,* 1984).   On the other hand, naturally occurring coagulants are usually presumed safe for human health.   Some studies on natural coagulants have been carried out and various natural coagulants were produced or extracted from microorganisms, animals, or plants (Kawamura*.,* 1991; Lee *et al.,* 1995; Ganjidoust *et al.,* 1997) .

Moringa oleifera is known as a tropical plant containing an active coagulating compound in the seeds. *M. oleifera* belongs to the family *Moringaceae* that is a single genus family of shrubs. Many studies (1983; Jahn, 1988; Muyibi and Okuofu, 1995; Ndabigengesere *et al.*, 1995; Muyibi and Evison, 1996) have been done on the performance of *M. oleifera* seeds as an alternative coagulant or a coagulant aid. However *M. oleifera* coagulant (MOC) has high coagulation activity only for high turbidity water. The activity is low for low turbid water (Muyibi and Evison., 1995). MOC has been found not to be effective as a natural coagulant for low turbid drinking waters but for high turbid waters in previous studies. If it is possible to enhance the coagulation activity of MOC, it could be used more widely for drinking water treatment. Our previous study (Okuda *et al.*, in press) improved extraction efficiency and enhanced coagulation capacity of MOC. We found that ionic strength of the extracting solution enhance the extraction efficiency of the active component from *M. oleifera* seeds. As the result, the coagulation capacity of MOC extracted by 1 M NaCl solution (MOC-SC) was 7.4 times as high as that of MOC extracted by distilled water (MOC-DW) for the removal of suspended kaolinite. A research group (Ndabigengesere and Narasiah, 1998) suggested that one of the shortcomings of MOC-DW was the increase in residual dissolved organic carbon (DOC) of the treated water. DOC is usually regarded as the source of odor, color and taste, and a precursor of disinfection by-products in drinking water treatment. Their results showed an increase in COD and E280 after the use of MOC. The increase, however, may have primarily resulted from inactive components of MOC-DW.

The objectives of this study are to purify the coagulation active component (MOC-SC-PC) of MOC-SC, and to study the coagulation characteristics of MOC-SC-PC. Coagulation activity in low turbid water and the residual DOC after coagulation were studied for MOC-SC-PC.

MATERIALS AND METHODS

Extraction of active component from *M. oleifera* seed.

M. oleifera seeds in dry pods were collected at Los Baños, Laguna, Philippines. The seeds were removed from the pods and were stored with winged seed covers at room temperature. The winged seed cover was shelled just before the extraction and the kernel was ground to a fine powder using a mortar and pestle. Suspended seed powder of 10 grams in 1 liter of 1.0 M NaCl solution was stirred using a magnetic stirrer for 10 minutes to extract active coagulation component. The solution was then filtered through a filter paper (5A, quantitative ashless; ADVANTEC) with about 7 μm of pore size (MOC-SC).

Purification of active component.

The active component (MOC-SC-PC) in MOC-SC was purified by dialysis, delipidation, then redissolved into a buffer followed by anion exchange as shown in Figure 1. One liter of MOC-SC was put into seamless cellulose dialysis tubing (Viskase Sales Corp) with molecular weight cutoff from 12 to 14 kDa and was placed in 10 liters of deionized water at a room temperature (about 20 °C). The external deionized water was changed several times for 2 days. White precipitate was produced in the dialysis tube by salting-out phenomenon. The precipitate was collected by centrifugation at 5,000 rpm for 20 min and was rinsed with deionized water. In the delipidation step, the precipitate was suspended in 500 ml of cold acetone and was homogenized using an homogenizer (Iuchi : DIGITAL-HOMOGENIZER) to remove lipids. The white precipitate was recovered from the cold acetone by centrifugation at 3,500 rpm for 30 min and was rinsed with cold acetone. The precipitate was dissolved into 1,000 ml of 0.1 M ammonium buffer (NH_4Cl-NH_3) at pH 10.5. Insoluble matter in the solution were removed by centrifugation at 3,500 rpm for 30 min. The solution was passed through an anion exchanger column (25 mm x 30 mm glass column packed with 12 ml of Amberlite IRA-900 equilibrated with 0.1 M ammonium buffer at pH 10.5). Active component on anion exchanger was eluted by increasing the ionic strength of NaCl solution passing through the column at a rate of 3 ml/min.
Purity of the MOC-SC-PC was verified through GPC analysis. A glass column packed with a gel (TOSO :

TSKgel TOYOPEARL HW-40, 31 x 550 mm) was used.   Five ml of the purified active component was fed into GPC column at a rate of 1 ml/min.   A moving buffer (0.1 M ammonium buffer) at pH 10.5 was used.

Synthetic turbid water.

Synthetic turbid water for coagulation tests was prepared by adding kaolin into tap water.   Ten grams of kaolin (CP grade, Katayama Chemical) was added to 1 liter of tap water.   The suspension was stirred for 1 hour for uniform dispersion of kaolin particle and then stood for 24 hours to allow for complete hydration of the particle.   The suspension was diluted to 200 ml using tap water to prepare synthetic turbid water with various turbidity just before coagulation test.

Coagulation test.

Jar test was used for the evaluation of coagulation.   Three hundred ml beakers filled with 200 ml of synthetic turbid water was placed on slot in the jar tester (SUGIYAMAGEN : NT-6).   Active components with different stages of purification was added into the beaker and was agitated at 150 rpm for 2 minutes, then the mixing speed was reduced to 30 rpm and was kept for 30 minutes. (Ndabigengesere and Narasiah, 1996)   After sedimentation for 1 hour, 5 ml of the sample was collected from the middle of the beaker and residual turbidity (RT_{sample}) was determined using a turbidimeter (ANA-148, Tokyo Photoelectric).   Turbidity was expressed as [$mg_{-kaolin}$ l^{-1}].   The residual turbidity of the coagulant-free kaolin suspension was also measured (RT_{blank}) as control.   Coagulation activity during the purification of active component in MOC-SC was calculated based on Lee's equation (Lee et $al.$, 1995).

$$\text{Coagulation Activity} = (RT_{blank} - RT_{sample}) / Rt_{blank}$$

Analytical methods.

The pH was measured using a pH meter (F-8, Horiba).   Protein was analyzed by the standard Lowry method (Clark and Switzer, 1976) and the protein assay method (Bio Rad).   Sugar was analyzed by the phenol-sulfuric acid method (Dubois et $al.$, 1956).   Dissolved organic carbon (DOC) was determined using a Total Organic Carbon Analyzer (TOC-500 and TOC-5000, Shimadzu).

RESULTS AND DISCUSSION

Purification of active component

The anion exchange chromatogram of pre-purified MOC-SC is shown in Figure 2.   Coagulation activity of each fraction was determined at a dose of 4 ml coagulant.   100 ml of pre-purified MOC-SC was passed through initially on the anion exchanger.   The 200 ml of 0.1 M ammonium buffer at pH 10.5 followed by NaCl gradient from 0.0 to 1.0 M with the same buffer was applied.

Three major peaks of DOC (Peaks 1,2,3) were obtained with the use of the NaCl gradient.   However, only the Peak2 showed coagulation activity.   To avoid contamination by Peak 1 and Peak3 components, another elution of Peak2 was attempted.   NaCl gradient from 0.1 to 0.4 M was used after the application of 500 ml of 0.1 M NaCl. The result is shown in Figure 3.   A single peak of DOC was obtained with high coagulation activity.

Purity of the purified active component (MOC-SC-PC, the peak in Figure 3) in MOC-SC was confirmed by the GPC analysis.   Five ml of MOC-SC-PC was added into GPC.   The result is shown in Figure 4.

MOC-SC-PC showed a sharp single peak on the GPC chromatogram and coagulation activity was coincident with the peek indicating purity of the active component. The molecular weight of the active component was estimated to be approximately 3,000 from polyethylene glycol standards (PEG 600, PEG 2000, PEG 6000). Molecular weight determined by dialysis membranes (Spectra / Por CE ; SPECTRUM with several pore sizes ranged from 0.1 to 10 kDa) was consistent with that estimated by the GPC analysis.

Characteristic changes in MOC-SC during the purification steps are summarized in Table 1. An arbitrary activity unit (Unit) is used in this section for the express of specific activity. The activity to decrease the turbidity from 50 mg-kaolin l^{-1} to 5 mg-kaolin l^{-1} in one liter is defined as one activity unit. Specific activity is the activity unit per mg DOC. Dialysis treatment increased specific activity by 26 times compared with the crude extract, MOC-SC. MOC-SC-PC had 8.93 Unit mg^{-1} of specific activity, and is 33.7 times as high as MOC-SC.

Coagulation active components of MOC-SC and MOC-DW

MOC-SC-PC has different characteristics from that extracted by distilled water (MOC-DW) as given in previous reports. The molecular weight of MOC-SC-PC was ca. 3,000 as shown in Figure 4, whereas MOC-DW was reported to be ca. 13,000 (Ndabigengesere *et al.,* 1995). Several studies have reported that the active component in MOC-DW is protein (Ndabigengesere *et al.,* 1995; Gassenschmidt *et al.*, 1991; Gassenschmidt *et al.*, 1995). However, MOC-SC-PC did not have absorbance at 280 nm, a characteristic wavelength for protein. It was also verified by the negative results of protein analysis using Lowry method and another protein assay methods (Bio-Rad). In addition, the polysaccharide analysis showed that the concentration of polysaccharide of MOC-SC-PC was nearly zero. It is not possible to have lipids in MOC-SC-PC because of the delipidation step in purification. Thus MOC-SC-PC is not protein, polysaccharide nor lipid, but an organic polyelectrolyte with molecular weight of ca. 3,000. IR data showed that the main functional group of MOC-SC-PC was amino group and hydroxide group, and MOC-SC-PC had little carboxyl group.

Coagulation activity of MOC-SC-PC

The coagulation activity of MOC-SC-PC was determined at various pH levels ranging from 2 to 12. pH was adjusted using 0.1N NH$_4$OH or 0.1N HCl immediately after the addition of MOC-SC-PC. As shown in Figure 5, MOC-SC-PC showed high coagulation activity at pH 8 or more and little coagulation activity was noted below pH 7.

Coagulation activity of MOC-SC-PC to low turbid water was evaluated using synthetic turbid water with 5, 10, 25 and 50 mg-kaolin l^{-1} at pH 9.0. MOC-SC-PC could reduce the turbidity in all the water tested down to less than 0.5 mg-kaolin l^{-1} at optimum dosage as shown in Figure 6.

Figure 7 shows the turbidity removal and residual DOC after coagulation using MOC-SC or MOC-SC-PC. Synthetic turbid water with 50 mg-kaolin l^{-1} was adjusted to pH 9.0. Residual turbidity decreased with an increase in the dosage of both coagulants. However, residual DOC increased even at the optimum dosage in case of MOC-SC. This is similar to the result of Ndabigengesere who studied MOC-DW (Ndabigengesere and Narasiah, 1998). On the other hand, MOC-SC-PC did not increase DOC. It is likely that the increase in DOC after coagulation by MOC-SC resulted from the inactive components for coagulation.

These coagulants were also applied to natural waters. Turbidity removal and residual DOC by MOC-SC and MOC-SC-PC at pH 9.0 for a polluted river water, Kurose River and an eutrophic lake water, Minaga Reservoir in Higashi-Hiroshima, Japan are shown in Figure 8 and Figure 9, respectively. Both MOC-SC and MOC-SC-PC were effective to remove turbidity in natural waters. Residual DOC concentrations showed the same trend as the synthetic water. MOC-SC increased residual DOC in the treated waters,

whereas MOC-SC-PC did not increase DOC.

CONCLUSIONS

The purpose of this study was to purify an active component of MOC-SC extracted by NaCl solution from *M. oleifera* seeds, and to determine the coagulation characteristics of the purified coagulation active component (MOC-SC-PC).   The specific conclusions derived from this study are as follows:

1) MOC-SC was purified by dialysis, delipidation and anion exchange.
2) The active component of MOC-SC (MOC-SC-PC) has no protein, polysaccharide and lipid.
3) The molecular weight of organic polyelectrolyte was ca. 3,000 and smaller than that of MOC-DW.
4) The optimum coagulation pH of MOC-SC-PC was pH 8 or higher.
5) Different from MOC-DW, MOC-SC-PC was effective for the treatment of low turbid water, with less than 5 mg-kaolin l^{-1} in kaolin.
6) MOC-SC-PC did not increase residual organic carbon concentration after coagulation.

ACKNOWLEDGMENT

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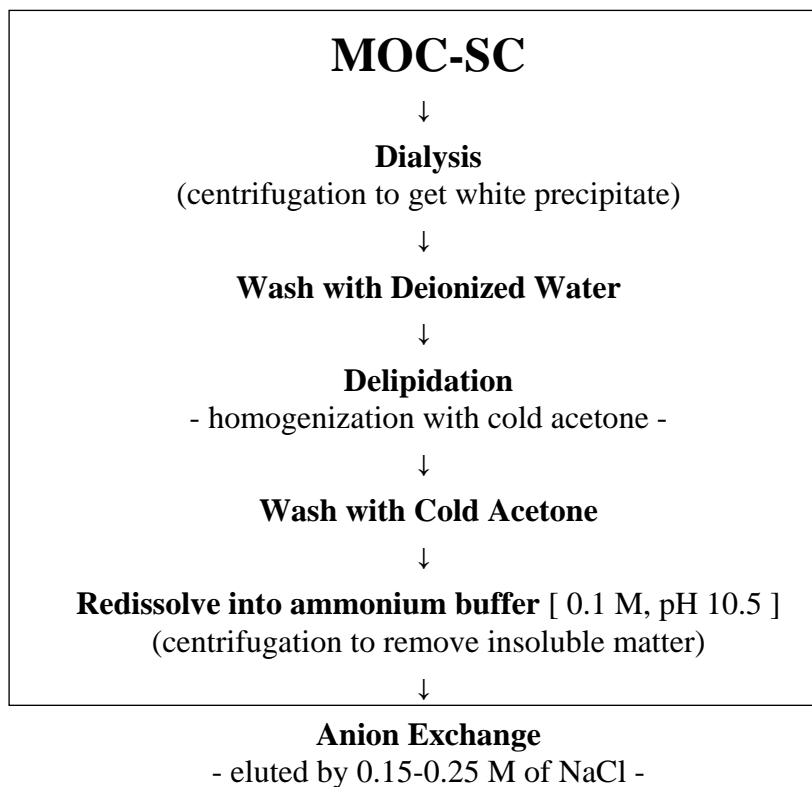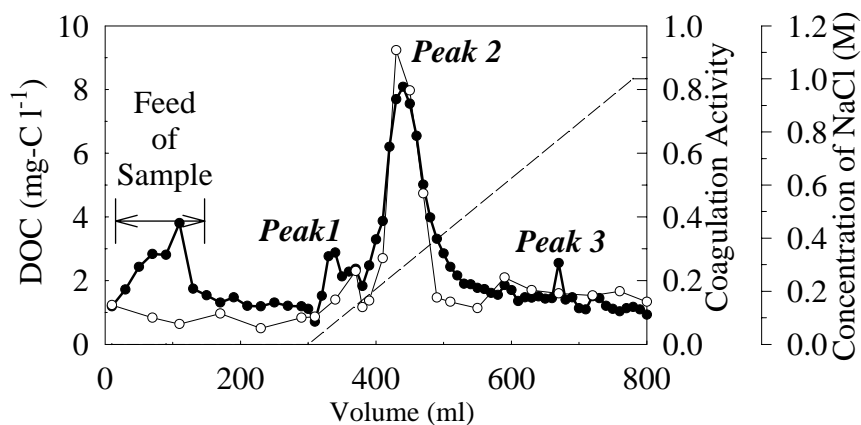
MOC-SC

↓

Dialysis
(centrifugation to get white precipitate)

↓

Wash with Deionized Water

↓

Delipidation
- homogenization with cold acetone -

↓

Wash with Cold Acetone

↓

Redissolve into ammonium buffer [0.1 M, pH 10.5]
(centrifugation to remove insoluble matter)

↓

Anion Exchange
- eluted by 0.15-0.25 M of NaCl -

Figure 1.   Purification Procedure

Figure 2.   Anion exchange chromatogram of pre-purified MOC. Open circles show coagulation
activities, closed circles show DOC and a dashed line shows NaCl gradient.
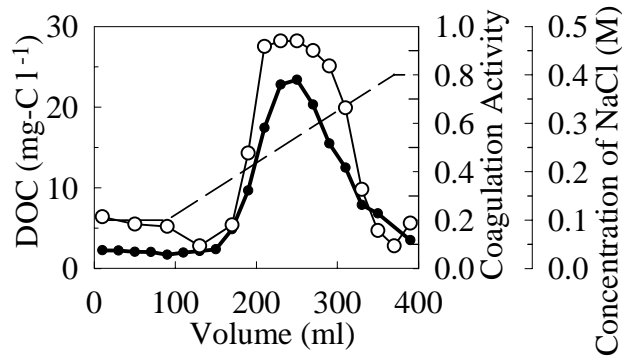
Figure 3.   Anion exchange chromatogram of pre-purified MOC.   Open circles show coagulation activities, closed circles show DOC and a dashed line shows NaCl gradient.
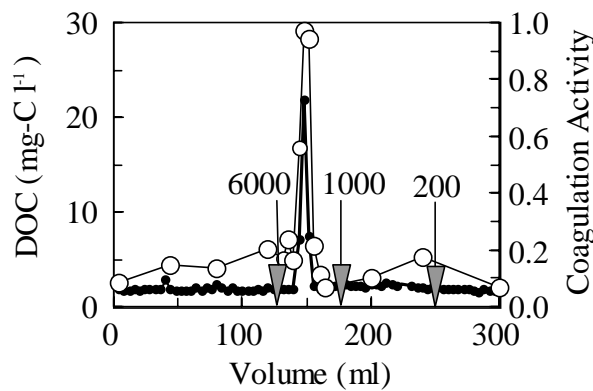
Figure 4. GPC chromatogram of the purified MOC.   Arrows indicate the volume for the three molecular makers.   Open circles show coagulation activities and closed circles show DOC.

Table 1.   Purification of MOC-SC. (1L sample)

Step	Activity (Unit)	DOC (mg-C)	Specific Activity (Unit mg^{-1})
Crude Extract	500	1880	0.265
Dialysis	51.3	7.40	6.91
Delipidation	50.0	6.80	7.40
Ion Exchange	58.5	6.60	8.93
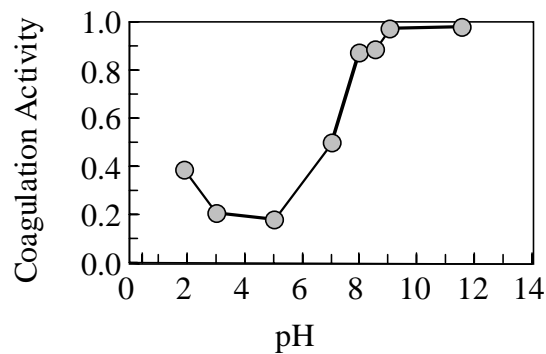
Figure 5.   Effects of pH on coagulation activity of MOC-SC-PC. (Dosage : 0.224 mg-C l^{-1})
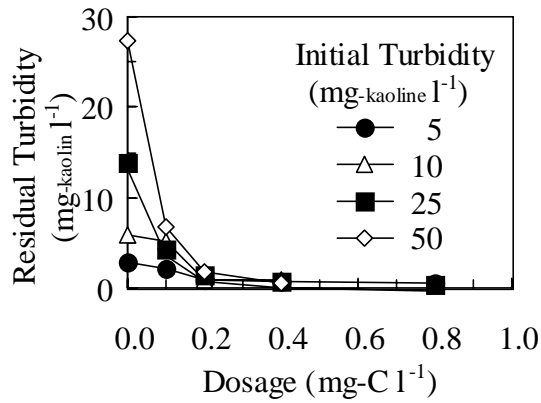
Figure 6. Effects of initial turbidity on residual turbidity after coagulation by MOC-SC-PC.
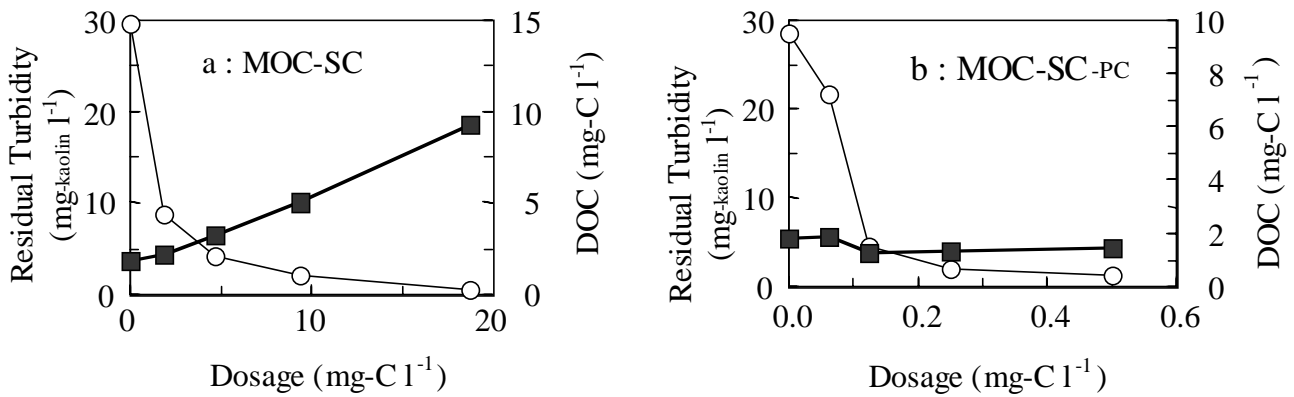

Figure 7. Residual turbidity and DOC of treated water by MOC-SC (a) and MOC-SC-PC (b) as coagulant for synthetic turbid water. (○ : turbidity, ■ : DOC)
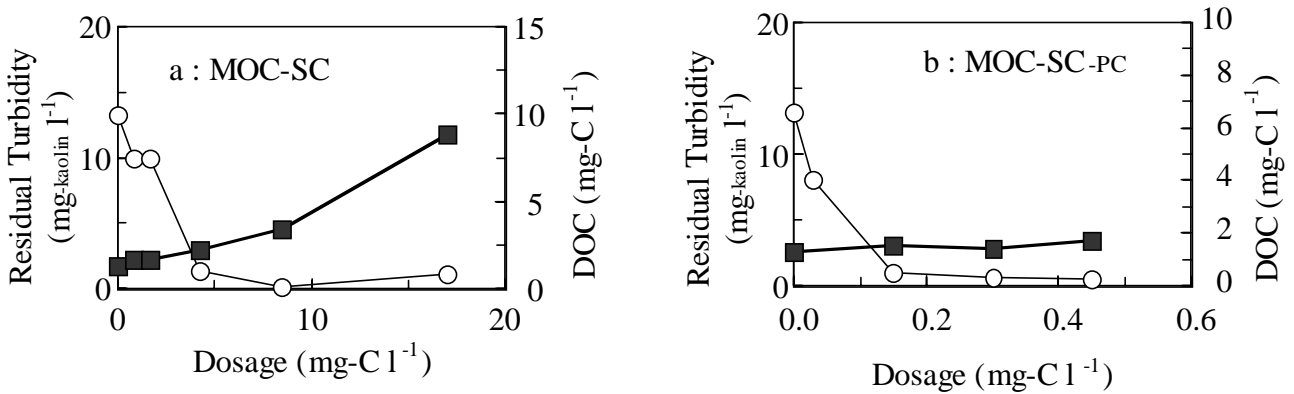

Figure 8. Residual turbidity and DOC by MOC-SC (a) and MOC-SC-PC (b) for a polluted river water (Kurose River, Higashi-Hiroshima, Japan). (○ : turbidity, ■ : DOC)
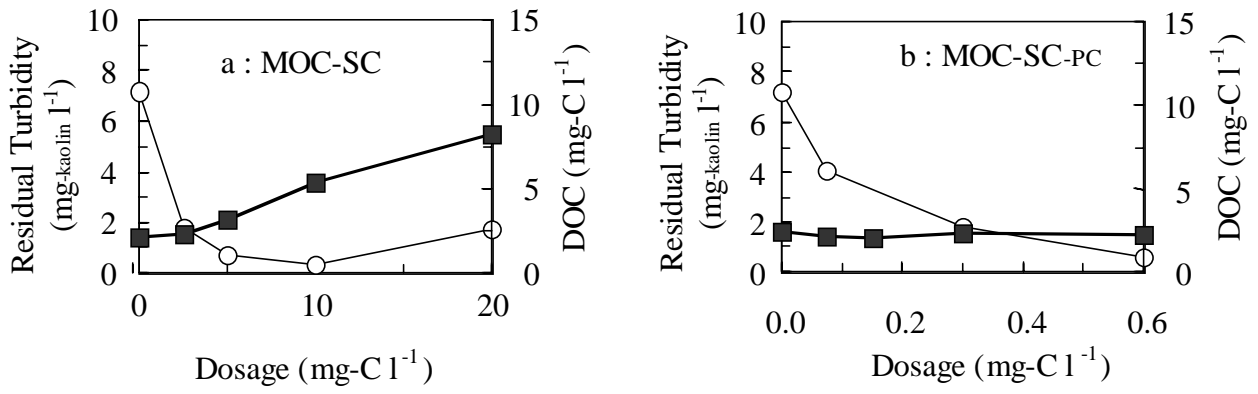
Figure 9. Residual turbidity and DOC by MOC-SC (a) and MOC-SC-PC (b) for an eutrophic lake water (Minaga Reservoir, Higashi-Hiroshima, Japan). (: turbidity,   : DOC)