J Incl Phenom Macrocycl Chem (2007) 57:21–27
DOI 10.1007/s10847-006-9168-0

ORIGINAL ARTICLE

Enhanced effect of RM-β-cyclodextrin on biodegradation of toluene in wastewater by activated sludge

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Received: 15 May 2006 / Accepted: 20 October 2006 / Published online: 18 January 2007

Abstract Recently, air and ground water pollution and contamination of soil by toluene have been drawing increasing attention and became an urgently important problem in environmental pollution. Hence, the development of highly sophisticated removal techniques of toluene is required for the global environmental preservation. Since toluene is a highly volatile material, it is difficult to treat it by usual activated sludge water treatment. In this study, in order to prevent volatilization of toluene, randomly methylated β-cyclodextrin (RM-β-CD) was used to complex with toluene and by reason of that, facilitates the biodegradation of toluene by activated sludge. The enhanced effect of RM-β-CD for the biodegradation of toluene by activated sludge was studied in batch systems. The addition of RM-β-CD dominantly promoted proliferation of activated sludge. This implied that the addition of RM-β-CD prevented toluene from evaporating during treatment, and as a result, toluene was effectively decomposed by the activated sludge.

Keywords RM-β-CD · Toluene · Activated sludge · Water pollution

Introduction

Toluene is one of the most important basic chemical compounds that have been used for manufacturing many chemical substances such as paints, plastics and others. Recently, air and ground water pollution and the contamination of soil by toluene have been drawing increasing attention and became an urgently important problem in environmental pollution. Hence, the development of highly sophisticated removal techniques of toluene is required for the global environmental preservation. Since toluene is a highly volatile material, it is difficult to treat it by usual activated sludge water treatment. In order to prevent volatilization of toluene, cyclodextrin was used to complex with it. One of the uses of cyclodextrins is to solubilize poorly soluble compounds. Hanna et al. [1, 2] studied the enhanced solubilization of hazardous compounds by randomly methylated β-cyclodextrin (RM-β-CD) at various pH and ionic strength. Trotta et al. [3] reported the effective removal of β-naphthol from wastewater by means of the increasing solubility of β-naphthol in RM-β-CD solution. Fava et al. [4] successfully used RM-β-CD for solubilization and bioremediation of PCB strongly adsorbed on the surface of soil. Neoh et al. [5] observed the analogously enhanced solubility of iodine in RM-β-CD solution. Cyclodextrins also have stabilizing effect on easily volatile compounds. Neoh et al. [5] and Yoshii et al. [6] have studied the evaporative stability of iodine and a few food flavors, and developed a new method for calculating the stability constant of binary host:guest systems from desorption curves.

For biodegradation of toluene, Reardon et al. [7] reported the degradation kinetics of toluene and its mixtures with other substances such as benzene and phenol by *Pseudomonas putida* F1. Parales et al. [8] investigated the bioremediation of toluene and trichloroethylene. The degradation pathway of toluene

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by *Pseudomonas putida* F1 was well documented by many researchers [9, 10]. Cyclodextrin-enhanced degradation of toluene by *Pseudomonas putida* was subjected by Schwarts et al. [11] with the use of β-cyclodextrin. One of the most effective and economical biodegradation methods for industrial wastewater is biological oxidation by means of activated sludge. Oláh et al. [12] carried out biodegradation of industrial wastewater in the presence of β-cyclodextrin in activated sludge system. They concluded that the enhancement of degradation rate was mainly due to the formation of the inclusion complex, resulting in the suppression of toxicity. Yoshii et al. [13, 14] studied the bioremediation of model biphenyl-contaminated soils with RM-β-CD and HP-β-CD by kneading with a twin-screw kneader. Biphenyl was effectively removed from the surface of the soil by the addition of RM-β-CD. The biphenyl in wastewater could be easily degraded in the presence of RM-β-CD by activated sludge.

In this study, the enhanced effect of RM-β-CD for the biodegradation of toluene by activated sludge was studied in batch systems. The increase of solubility and the reduction of vaporization of toluene in the presence of RM-β-CD were investigated. The effects of step disturbance of RM-β-CD concentration on degradation of toluene were also examined.

Experimental

Materials

Randomly methylated β-CD (RM-β-CD) with degree of substitution (DS) of 1.6–1.9 per anhydro glucose unit was purchased from Cyclochem Co., Ltd. (Kobe, Japan). RM-β-CD was subjected to vacuum drying at 90 °C for 24 h prior to use. Diammonium hydrogenphosphate and toluene was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Activated sludge was obtained from a municipal wastewater treatment plant in Osaka, Japan. Unless otherwise noted, all the chemicals used were of analytical reagent grade.

Solubility of toluene in RM-β-CD solution of various concentrations

Powder of RM-β-CD was weighed and dissolved with distilled water in a 50 mL volumetric flask to obtain RM-β-CD solutions with concentrations within the range of 0.763–5.34 mM. Subsequently, *ca.* 2 g of toluene was added into the flask which was then shaken in a bioshaker (BR-15LF, Taitec Corp., Saitama, Japan) at 160 rpm, 25 °C for 44 h. This time length was considered sufficient to reach equilibrium. After 44 h, 2 g of the saturated solutions of toluene were transferred into a glass bottle and then chloroform (containing of 100 ppm decane as an internal standard) of an amount equal to the sample solution was added before the bottle was tightly capped. The solution was heated in a water bath for 20 min at 80 °C to extract toluene into the chloroform. During heating, the tube was periodically shaken with a vortex mixer. After extraction, the samples were centrifuged in a Kubota 2010 centrifuge (Kubota Inc., Tokyo, Japan) for 20 min at 3,000 rpm to separate water and chloroform phases. The concentration of toluene in the chloroform was determined by FID gas chromatography (GC-17A, Shimadzu, Kyoto, Japan). The sample was separated on an HR-1 column (Shimadzu) with helium as carrier gas at 2.5 mL/min. The column temperature was programmed as: the initial temperature was set at 70 °C for 1 min, and then temperature was increased at a rate of 40 °C/min to a final temperature of 250 °C and held for 1 min. The injector and detector temperature were 280 °C and 150 °C, respectively.

Desorption experiment of toluene

Toluene solution with concentration of 100 ppm was prepared by weighing 0.1 g of toluene and mixing with 1,000 ml of distilled water. RM-β-CD of 0, 17.78, and 35.56 g were dissolved into the 200 g toluene solution and filled up to 250 mL in volumetric flask. The RM-β-CD concentrations corresponded respectively to 0, 50, and 100 molar folds the toluene concentration of 100 ppm. The solution was poured into a 500 mL tall beaker and put in a water bath of 30 °C. Air was bubbled into the beaker at 50 mL/min to desorb toluene from the solution. At prescribed time intervals, 1.5 mL of the solution was sampled and the retention of toluene was measured by gas chromatography. The analytical procedure of toluene was the same as described above. According to the estimation procedure developed by Neoh et al. [5], the stability constant of toluene could be estimated with the retention time course of toluene in the solution.

Measurements of biodegradation of toluene by activated sludge under various RM-β-CD concentrations

The effect of RM-β-CD concentration on the biodegradation of toluene was measured under various concentrations of RM-β-CD in a buffer solution. Diammonium hydrogenphosphate buffer solution (20 mM, pH 6.0) was prepared and half a liter of it was put into a 1,000 mL Erlenmeyer flask. After being autoclaved at 121 °C for 20 min, ca. 40 mL of the solution was added with 0, 1.78, 3.55, and 7.11 g of RM-β-CD respectively and filled up to 50 mL in volumetric flasks. These concentrations of RM-β-CD were equivalent to 0, 25, 50, and 100 M folds the concentration of 100 ppm toluene respectively. The solutions were put into 100 mL Erlenmeyer flasks and approximately 0.2 g of activated sludge was put in the solutions, followed by the addition of toluene (0.005 g). The flasks were then shaken at 120 rpm, 30 °C. At every 24 h, 1.5 mL of the solution was sampled and measured its absorbance at 600 nm with a spectrophotometer (V-560, Nihonbunko, Tokyo, Japan) as the concentration of activated sludge. After sampling, 1.5 mL of the RM-β-CD solution containing 100 ppm toluene was added to the Erlenmeyer flask to continue incubation. The concentration of toluene in the sample solution was measured by an analogous procedure mentioned above. To 1 mL of the sampled solution, an equal amount of hexane (containing of 100 ppm decane as an internal standard) was added to extract the toluene at 90 °C for 30 min. One microliter of the extract (hexane) was injected to a GC-MS (GSMS-QP5050, Shimadzu, Kyoto, Japan) to quantify toluene. The separation was performed on an HR-1 column (Shimadzu) with temperature programmed from 70 to 200 °C at the rate of 20 °C/min. The carrier gas was helium, flowing at the rate of 1.5 mL/min. The sampling time was 0.2 min in split-less mode.

Biodegradation of toluene by activated sludge on step disturbance of RM-β-CD concentration

The effects of step disturbance of RM-β-CD on the growth of activated sludge and the biodegradation behavior of toluene were measured. In the experiment of step disturbance of RM-β-CD concentration, for the first 4 days, the activated sludge was incubated at 100 ppm of toluene in a similar procedure mentioned above, but without RM-β-CD. After 4 days, RM-β-CD of 1.78, 3.55, and 7.11 g were dissolved in the buffer solutions to induce a sudden increase in RM-β-CD concentration. After every sampling in the experiments, 1.5 mL of buffer solution with corresponding concentrations of RM-β-CD was added to replenish the sampling loss. The concentration of activated sludge was measured by the absorbance at 600 nm with the V-560 spectrophotometer, and the concentration of toluene with GSMS-QP5050.

Results and discussion

Enhancement of toluene solubility by addition of RM-β-CD

The enhancement of toluene solubility by addition of RM-β-CD is illustrated in Fig. 1, where the concentration of toluene in aqueous solution was plotted against the concentration of RM-β-CD in the solution. The toluene concentration is linearly increased with the increase of the RM-β-CD concentration, indicating that the solubility of toluene was enhanced by RM-β-CD. The apparent stability constant can be calculated as 439 M^{-1} by the slope and the intercept of the linear regression line with the ordinate in Fig. 1

Influence of RM-β-CD on desorption of toluene

Figure 2 shows the effects of RM-β-CD concentration on the desorption time courses of toluene. It was found that the desorption of toluene was markedly inhibited by the addition of RM-β-CD. This implies that the inclusion complex of toluene with RM-β-CD might have occurred readily and hence increased the toluene stability in aqueous phase. Based on several assumptions, Neoh et al. [5] have derived a useful equation for estimation of apparent stability constant between guest compound and cyclodextrin. The first linear section in the retention curve in Fig. 2 was used for determination of the apparent stability constant. The reciprocal of the apparent volumetric mass transfer coefficient

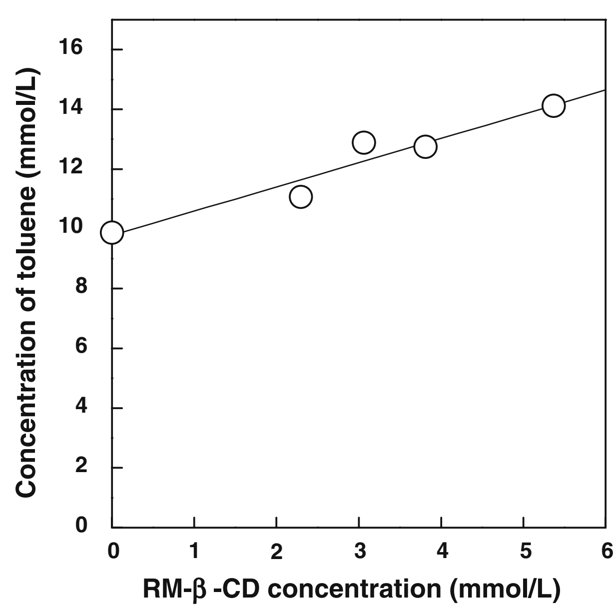

Fig. 1 The enhancement of toluene solubility by the addition of RM-β-CD

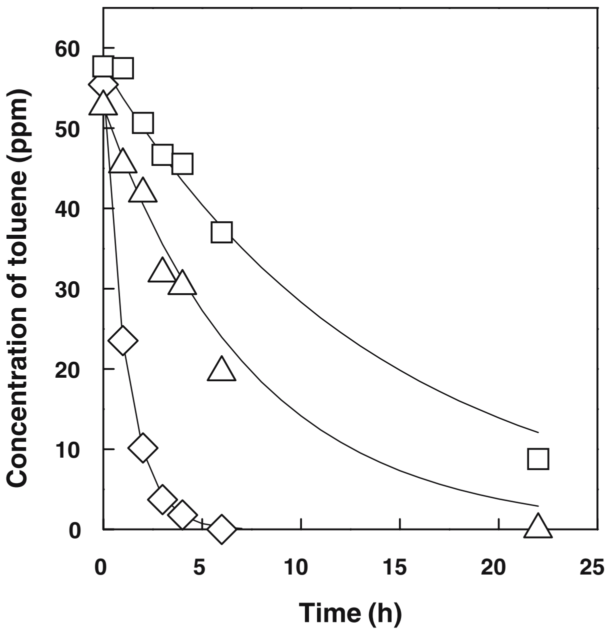

Fig. 2 The effects of RM-β-CD concentration on the desorption time courses of toluene. RM-β-CD concentration (molar folds to toluene (100 ppm)):◇ 0, △ 50, □ 100. Incubation temperature: 30 °C

$(k_La)_{app}$ versus the concentration of RM-β-CD could satisfactorily be linearly correlated as shown in Fig. 3. This means that the apparent stability constant can be computed by dividing the slope by the intercept on the y-axis. The apparent stability constant obtained was 235 M^{-1}. This value is a little lower in comparison to the one estimated from solubility method. This may be due to the derivation of equation based merely on mass transfer theory [5, 6].

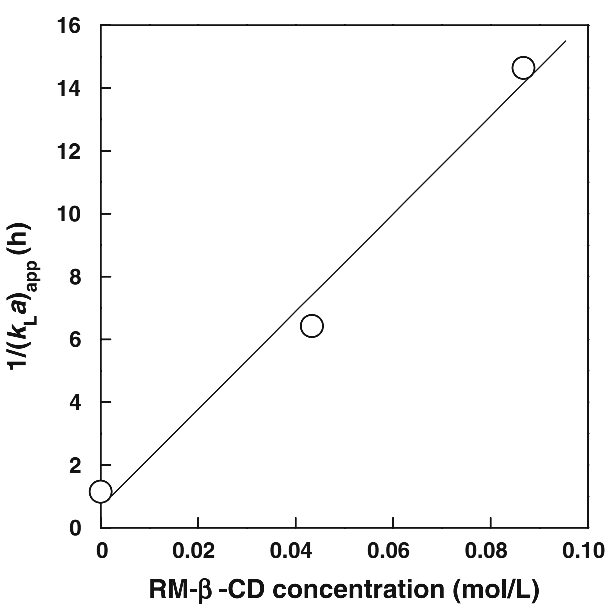

Fig. 3 The reciprocal of the apparent volumetric mass transfer coefficient $(k_La)_{app}$ vs. the concentration of RM-β-CD

Enhanced effect of RM-β-CD on the growth of activated sludge and biodegradation of toluene

Figure 4 shows the time courses of the growth of activated sludge at toluene concentration of 100 ppm for different RM-β-CD contents. The growth of activated sludge, which was measured by the absorbance at 600 nm, was clearly enhanced with the increase in the RM-β-CD content. The growth curves of the activated sludge were analyzed with the following Verlhurst Eq. (1).

$$\frac{A_{600}}{A_{600}^{max}} = \frac{1}{1 + a\exp(-kt)} \tag{1}$$

where A_{600} is the absorbance at 600 nm, A_{600}^{max} is its maximum values, k is the growth rate constant, t is time, and a is a constant defined as follow:

$$a = \frac{A_{600}^{max} - A_{600}^{0}}{A_{600}^{max}} \tag{2}$$

where A_{600}^{0} is the initial absorbance at 600 nm. The growth rate constant k was a function of RM-β-CD concentration as shown in Fig. 5. The value of k

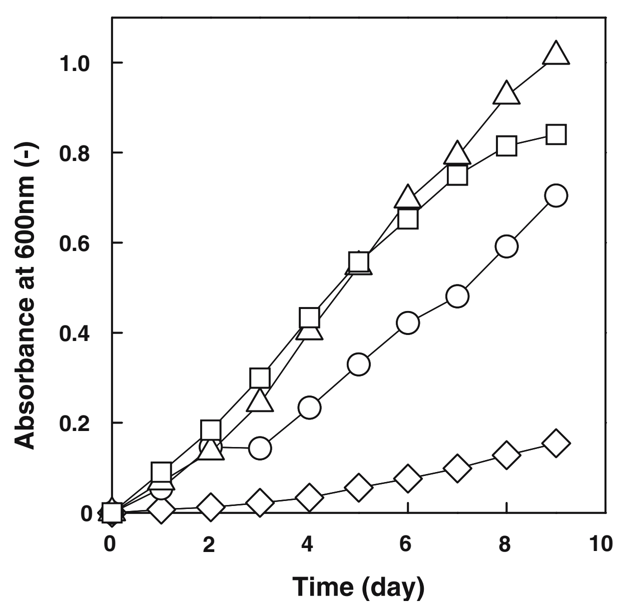

Fig. 4 The time courses of the growth of activated sludge at toluene concentration of 100 ppm for different RM-β-CD contents. RM-β-CD concentration (molar folds to toluene (100 ppm)):◇ 0, ○ 25, △ 50, □ 100. Incubation temperature: 30 °C

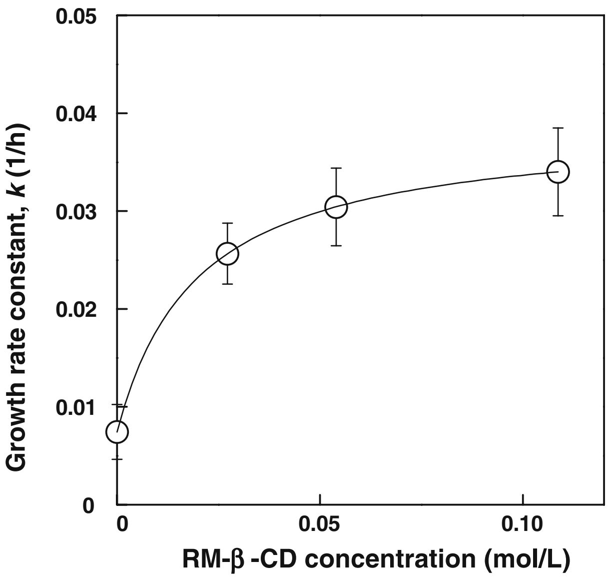

Fig. 5 Effect of RM-β-CD concentration on the growth rate constant estimated by Verlhurst equation. Incubation temperature: 30 °C. Toluene concentration: 100 ppm

increased with the increase of RM-β-CD content. The functional relationship could be correlated by the following equation.

$$k = \frac{1.48 \times 10^{-4} + 0.039[CD]}{0.02 + [CD]} \quad (3)$$

where $[CD]$ is the concentration of RM-β-CD in molar (M).

The biodegradation time courses of toluene are shown in Fig. 6(a) for four different RM-β-CD concentrations. In Fig. 6(b), the concentrations of toluene without activated sludge are illustrated for different RM-β-CD concentrations. The differences of these two groups of toluene concentrations for the corresponding RM-β-CD concentrations would be equal to the amount of toluene decomposed by the activated sludge. It could be observed that the biodegradation was markedly enhanced by the addition of RM-β-CD. The useful effect of RM-β-CD was caused by both its ability to inhibit toluene desorption and the diminution in toxicity of toluene due to the formation of the inclusion complex.

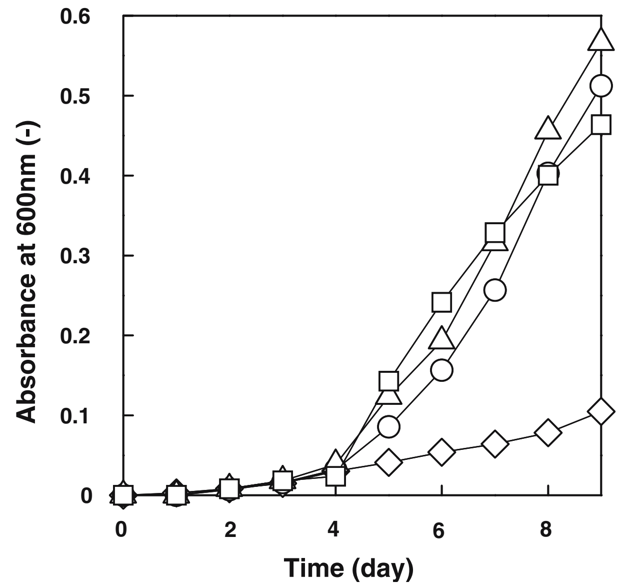

Fig. 7 Effects of step disturbance of RM-β-CD concentration on the growth of activated sludge. The RM-β-CD concentration was abruptly increased from 0 to 25, 50, and 100 molar folds the concentration of toluene (100 ppm) at the end of the fourth day. RM-β-CD concentration (molar folds to toluene (100 ppm)): ◇ 0, ○ 25, △ 50, □ 100. Incubation temperature: 30 °C

Effects of step disturbance of RM-β-CD concentration on the growth of activated sludge and the biodegradation of toluene

In order to make clear the effect of RM-β-CD, the concentration of RM-β-CD was changed abruptly during the toluene biodegradation treatment. The time course of the growth of activated sludge is shown in Fig. 7. The activated sludge was cultured for the first

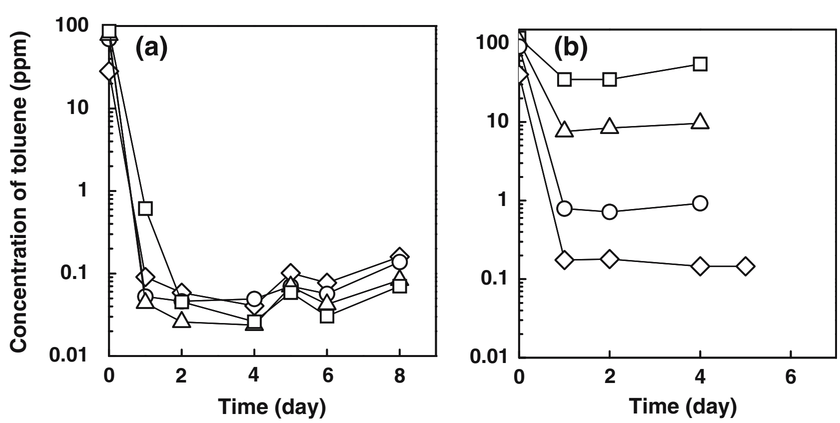

Fig. 6 The concentration time courses of toluene in the presence (**a**) and the absence (**b**) of activated sludge. RM-β-CD concentration (molar folds to toluene (100 ppm)): ◇ 0, ○ 25, △ 50, □ 100. Incubation temperature: 30 °C

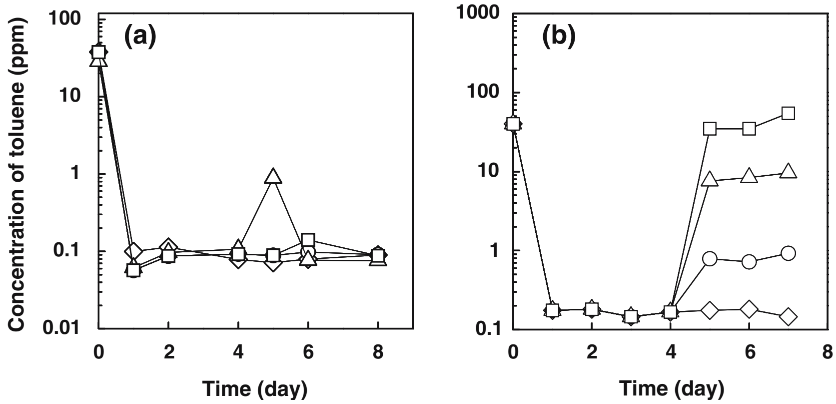

Fig. 8 The concentration time courses of toluene in the presence (**a**) and absence (**b**) for step disturbance of RM-β-CD concentration. The RM-β-CD concentration was increased from 0 to 25, 50, and 100 molar folds the concentration of toluene (100 ppm) at the end of the fourth day. RM-β-CD concentration (molar folds to toluene (100 ppm)): ◇ 0, ○ 25, △ 50, □ 100. Incubation temperature: 30 °C

4 days at 100 ppm toluene concentration without RM-β-CD. At the end of the fourth day, RM-β-CD was added to the culture solutions at concentrations of 25, 50 and 100 M folds the concentration of toluene (100 ppm). As observed in Fig. 7, in the first four days, the activated sludge could not be grown at all, indicated by low absorbance at 600 nm. However, after that, the absorbance increased sharply except for the absence of RM-β-CD, indicating that the activated sludge proliferated with the addition of RM-β-CD. The growth time courses of different RM-β-CD concentrations were nearly the same and independent of the amount of RM-β-CD. The concentrations of toluene in the culture solutions with step disturbance of RM-β-CD content are shown in Fig. 8 for in the presence (a) and the absence (b) of activated sludge. In the case of absence of activated sludge, the toluene concentration increased abruptly with the addition of RM-β-CD because of the formation of inclusion complexes. However, the abrupt increase of the included toluene was successfully dwindled by the activated sludge as shown in Fig. 8(a).

Conclusion

The enhanced effect of RM-β-CD for the biodegradation of toluene by activated sludge was studied in batch systems. RM-β-CD successfully retained toluene by preventing it from evaporating. The ability to impede desorption of toluene was remarkable at high RM-β-CD contents. RM-β-CD also dominantly increased the activated sludge concentration and enhanced the biodegradation of toluene in the solutions. This implied that the addition of RM-β-CD prevented toluene from evaporating during incubation, and as a result, toluene was effectively decomposed by the activated sludge due to the formation of inclusion complex.

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