Journal of Fluorine Chemistry 135 (2012) 350–353

Contents lists available at ScienceDirect

Journal of Fluorine Chemistry

journal homepage: www.elsevier.com/locate/fluor

Short communication

Retransformation of fluoride ion generated from the biodegradation of benzotrifluoride to calcium fluoride

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ARTICLE INFO

Article history:
Received 6 July 2011
Received in revised form 26 July 2011
Accepted 27 July 2011
Available online 19 August 2011

Keywords:
Fluorine resource
Biodegradation
Calcium fluoride
Fluoride ion
Retransformation

ABSTRACT

The retransformation of fluoride ion generated from biodegradation of benzotrifluoride to calcium fluoride, was described. For the effective use of this process, the recovery and reuse of degradation ability of *Rhodococcus* sp. were described.

1. Introduction

In the fluorine science, the recycle of fluorine resource is considered as priority in order to contribute the sustainable development [1,2]. Until now, fluorite is consumed by two industries: chemical industry, for the manufacture of hydrofluoric acid, and the iron and steel industry. The common treatment to remove fluoride ion from industrial wastewater is chemical precipitation [3–8]. It is an important factor to reuse fluoride ion generated from the degradation and/or biodegradation of used fluorinated materials from the sustainable developmental point of view.

While fluorinated materials are most stable compounds in the environment, nature seems to be reluctant to develop biological degradation [9–13]. We have reported the transformation of 2,2-difluoroethanol to difluoroacetic acid and the accumulation of difluoroacetic acid [14].

Recently, we have reported the isolation of bacteria which was possible to accumulate fluorinated materials [15], and the recycle of fluorine resource generated from the degradation or biodegradation of fluorinated materials, producing 5′-FDA and 5-FDR by the enzymatic processes [16,17]. Furthermore, we have reported that the concentration of fluoride ion when the biodegradation was carried out at 28 °C for 8 day aerobically in the test tube, was up to a few mM in the time course of the production for fluoride ion [15,16]. However, in general, fluorinated chemicals are prominent xenobiotics and have low biodegradability due to their high stability. Consequently, these materials have received much less attention on the accumulation and biodegradation by bacteria, fungi and yeasts in the environment. Furthermore, it is not clear the circulatory of fluoride ion generated from the biodegradation of used fluorinated materials.

On the basis of the greenery chemistry, it is of great importance to establish processes to retransform fluoride ion generated from the biodegradation of used fluorinated materials to calcium fluoride that are required both now and in the future for the fluorine source of fluorinated materials.

For our continuous studies on the recycle of fluorine resource based on the biotechnology, we would like to describe the production of calcium fluoride which can be one of the processes for the recycle of fluorine resource in a biological way. Especially, in attempting to develop the continuous generation of fluoride ion, we describe the restoration and reuse of degradation ability of *Rhodococcus* sp.

2. Results and discussion

It is well known that calcium fluoride is an important fluorine resource for the preparation of fluorinated materials in industrial scale [18,19]. In the sustainable development of fluorine science, the recycle of fluoride ion generated from the degradation and/or biodegradation of fluorinated materials, is one of the most important research works. For this purpose, we have examined

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doi:10.1016/j.jfluchem.2011.07.026

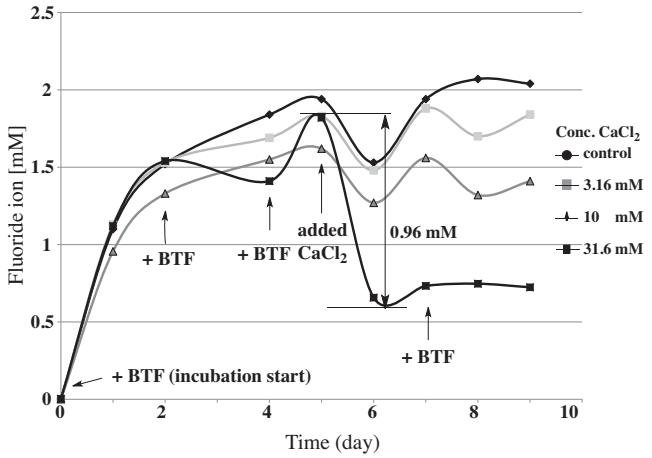

Fig. 1. The time course for the retransformation of fluoride ion after adding calcium chloride.

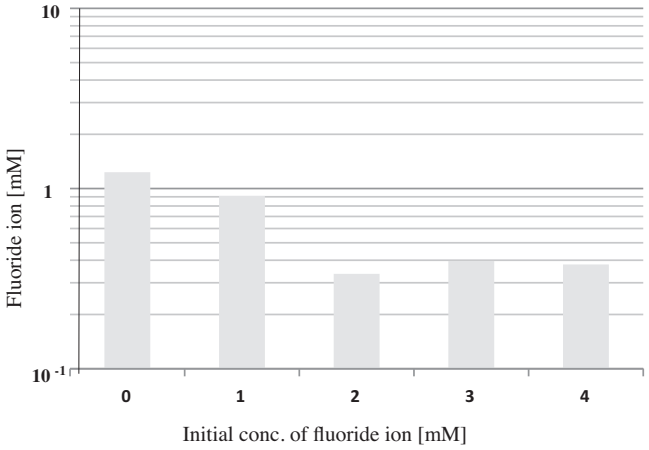

Fig. 3. The effect of fluoride ion on the activity of bacterial culture.

the following system to retransform the fluoride ion as the precipitate of calcium fluoride from calcium chloride and fluoride ion generated from biodegradation of benzotrifluoride by *Rhodococcus* sp.

After calcium chloride added into the cultivation layer based on the biodegradation of BTF by *Rhodococcus* sp., we have checked the time course of production of fluoride ion. The time course as shown in Fig. 1 supports that the concentration of fluoride ion after adding BTF at 4th day increases, and that it decreases smoothly together with the preparation of calcium fluoride after adding calcium chloride at 5th day after starting the incubation. The decreased peaks arised from the addition of calcium chloride suggest that calcium fluoride is produced from the reaction of calcium chloride and fluoride ion generated from the cultivation layer based on the biodegradation of BTF by *Rhodococcus* sp. To detect the amount of the generated calcium fluoride, we have examined the following procedure in the above incubation system. After adding calcium chloride into the above cultivation layer at 5th day after starting the incubation, the produced calcium fluoride in the incubation system was transferred to the distilled water, and then the concentration of fluoride ion in the supernatant was detected by ISE combination fluoride and/or ion chromatography system (DIONEX: ICS-2100; column. IonPac AS19). Furthermore, we have found that the concentration of calcium chloride affects on the improvement of calcium fluoride precipitate as shown in Fig. 2. In the case of $CaCl_2$ concentration (31.6 mM), we have found that the concentration of fluoride ion decreased 0.96 mM after adding calcium chloride as shown in Fig. 1, and that the fluoride ion generated from the degradation of benzotrifluoride by *Rhodococcus* sp. was recovered in calcium fluoride (0.64 mM) as shown in Fig. 2. The above results suggest that the generated fluoride ion is retransformed to calcium fluoride up to 67%.

Furthermore, we have found that the concentration of fluoride ion after adding BTF (20 mM) at 7th day was not recovered until the same concentration before the addition of calcium chloride as shown in Fig. 1. To make clear the above reasons, we have examined the relationship between the biodegradation of BTF by *Rhodococcus* sp. and the concentration of fluoride ion and/or calcium chloride. We have found that the increase of concentration of fluoride ion and/or calcium chloride affected on the degradation ability of *Rhodococcus* sp. as shown in Figs. 3 and 4. To make the above route useful as the calcium fluoride production for the recycle of fluorine resource circulatory system, we have examined the recovery and reuse of degradation ability of *Rhodococcus* sp.

For the recovery and reuse of the above mentioned ability, the cells containing *Rhodococcus* sp. were collected and removed by centrifugation at 5th day after starting the incubation. Into the residual cells containing *Rhodococcus* sp., the medium (4 ml), and BTF (20 mM) were added, and then the culture fluid was incubated

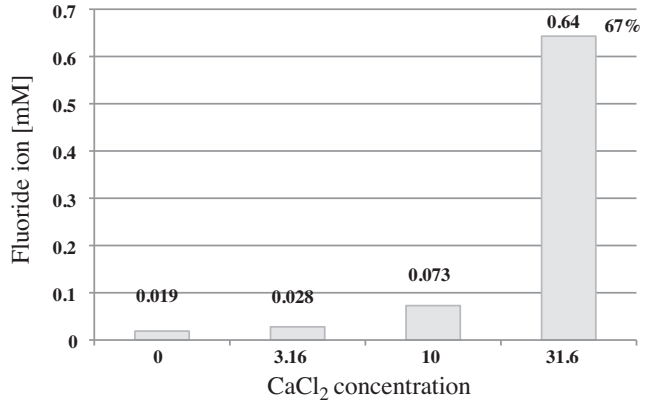

Fig. 2. The concentration of fluoride ion based on the calcium fluoride generated from the different amounts of calcium chloride.

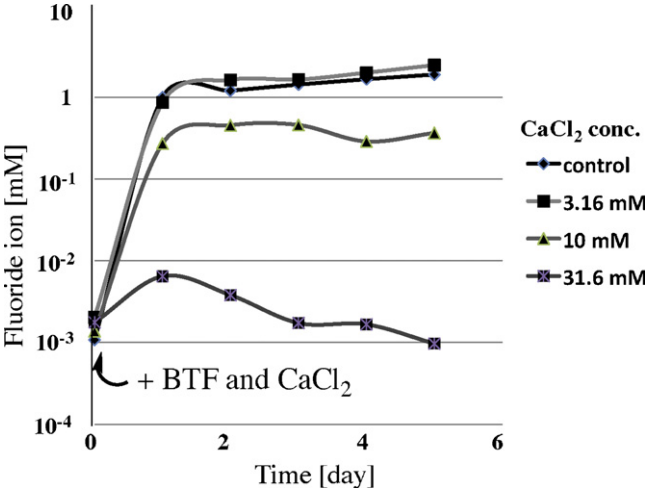

Fig. 4. The effect of conc. $CaCl_2$ on the activity of bacterial culture.

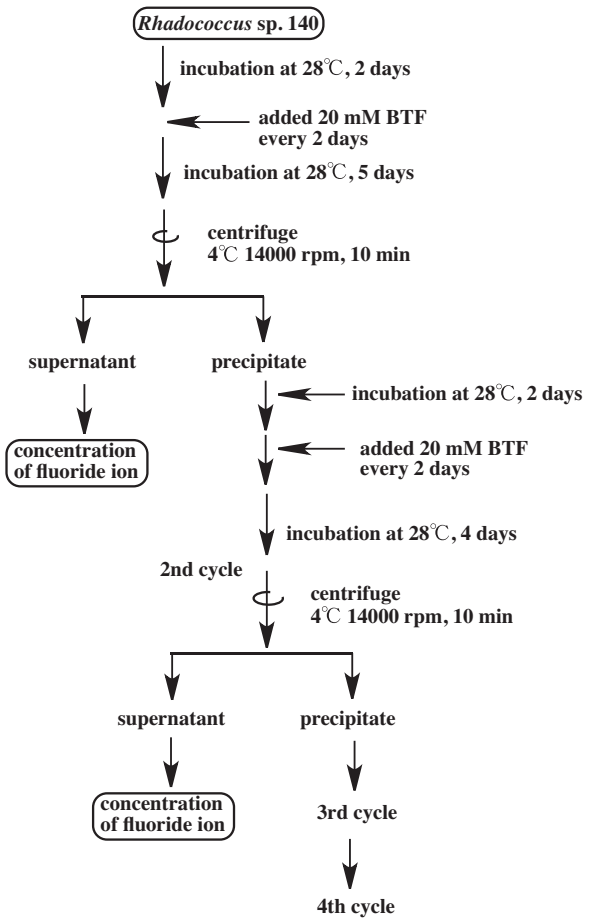

Fig. 5. Restoration of *Rhadococcus* sp. function.

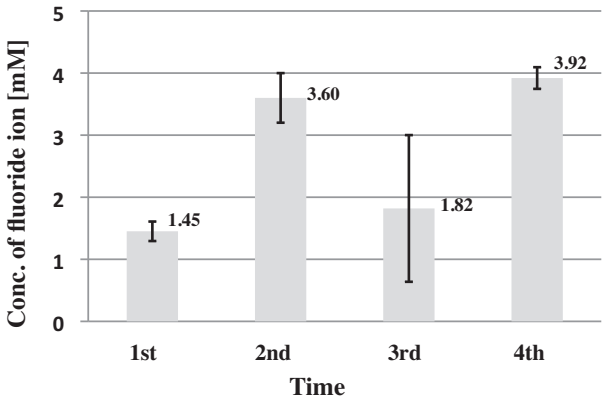

Fig. 6. Conc. of fluoride ion in every cycle.

again (the same procedure was carried from second to fourth cycles) as shown in Fig. 5. From the concentration of fluoride ion in supernatant of each as shown in Fig. 6, we have found that the recovered *Rhodococcus* sp. was possible to reuse for the degradation of BTF to produce the fluoride ion, and that the retransformation of the generated fluoride ion to calcium fluoride was proceeded smoothly.

3. Conclusion

We have established the route to calcium fluoride *via* the retransformation of the fluoride ion generated by the biodegradation of BTF with *Rhodococcus* sp. as one of the processes for the recycle of fluorine resource in order to contribute the sustainable development. We have succeeded the recovery and reuse of degradation function of *Rhodococcus* sp. for the effective use of this process as the recycle of fluorine resource circulatory system. We have found that the generated fluoride ion is recovered in calcium fluoride up to 67%.

4. Experimental

4.1. Bacterial strain

Rhodococcus sp. strain was characterized and identified in our reported literature [15].

4.2. Procedure for the production of calcium fluoride

Into a test tube production medium (15 ml) derived from starch (0.5%), sucrose (0.5%), N.Z. Amine (0.25%), polypeptone (0.25%), yeast extract (0.2%), extract ehlich (0.1%), KH_2PO_4 (0.1%), $MgSO_4{\cdot}7H_2O$ (0.05%), 150 μl of *Rhodococcus* sp. culture was inoculated. Into the above test tube, BTF (20 mM × 4 times) was added, and then the degradation of benzotrifluoride was carried out at 28 °C aerobically. After adding calcium chloride into the above cultivation layer at 5th day after starting the incubation, the produced calcium fluoride in the incubation system was transferred to the distilled water, and then the concentration of fluoride ion in the supernatant was detected by ISE combination fluoride and/or ion chromatography system (DIONEX: ICS-2100; column. IonPac AS19).

4.3. Recovery and reuse of the defluorination ability of Rhodococcus sp.

Into a test tube production medium (4 ml) derived from starch (0.5%), sucrose (0.5%), N.Z. Amine (0.25%), polypeptone (0.25%), yeast extract (0.2%), extract ehlich (0.1%), KH_2PO_4 (0.1%), $MgSO_4{\cdot}7H_2O$ (0.05%), 150 μl of *Rhodococcus* sp. culture was inoculated. Into the above test tube, BTF (20 mM × 3 times) was added, and then the degradation of benzotrifluoride was carried out at 28 °C for 5 days aerobically. Cells were collected and removed by centrifugation. Into the residual cells containing *Rhodococcus* sp., the above medium (4 ml), and BTF (20 mM) were added, and then the culture fluid was incubated again (the recovered precipitates containing *Rhodococcus* sp. were used in the second cycle. the same procedure was carried from second to fourth cycles).

The concentration of fluoride ion in supernatant of each was detected by ISE combination fluoride (ION pH/mV/ORP; Mettler-Toledo Group, Swiss) and/or ion chromatography system (DIONEX: ICS-2100; column. IonPac AS19).

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