ISSN: 2338-1345 – Vol. 2 (2): 5-8 2014 Journal online http://journal.bakrie.ac.id/index.php/APJSAFE

Asia Pacific Journal of Sustainable Agriculture Food and Energy (APJSAFE)

Selenium Recovery and Conversion by a Filamentous Fungus, *Aspergillus oryzae* Strain RIB40

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Abstract—A filamentous fungus (koji mold), *Aspergillus oryzae* strain RIB40 could be applied for selenium recovery and conversion. The granular cell-aggregates (mycelial cells) were able to reduce and convert selenite into nano-sized elemental selenium depositions when the granules were incubated under aerobic condition shaking at 120 rpm with a 100 ml baffled Erlenmeyer flask contained 20 ml of PD (potato dextrose) medium. In the aerobic condition, selenite (1 mM) was removed completely (over 99 %) from the culture within 24 hours by the mycelial cell-granules of strain RIB40. The cells turned red-orange due to the formation of elemental selenium nanospheres. Results of total selenium quantity determination in the red-orange coloured cellular granules by the atomic absorption method showed that most of selenite which disappeared from the culture were recovered to the granular cell-aggregates. These results demonstrate that a filamentous fungus, *Aspergillus oryzae* strain RIB40 can be used as an effective bio-material for recovery and reduction, and conversion of selenium-oxyanion in bioremediation, elemental resource recycling, biomineralization and biofabrication.

***Keywords*— *Aspergillus oryzae*, Selenite reduction, Selenium recovery.**

INTRODUCTION

Selenium (Se) is an important rare element for production of various industrial materials such as semiconductor, rectifier, photocells, photographic tuner and coloured glass (Zhang, 2004; Haygarth, 1994). Se is also widely found and distributing in a variety of chemical forms in rocks, soil, freshwater and marine sediments, ground water and diverse organisms (Conde, 1997; Doran, 1982; Bodnar, 2012). This chalocogen element plays significant roles in aquatic and terrestrial ecosystem and biological metabolisms and components. Furthermore, from the nutrition side, selenium is proved to be an essential element for algae (Araie, 2009), plants (Sors, 2005), animals and human being (Rayman, 2012). Nowadays, it has become that we can easily purchase the selenium supplements as "selenium yeast" (Kieliszek, 2013; Schrauzer, 2006) tablets in a drugstore in our daily life.

Meanwhile, its soluble oxy-anionic form such as selenate and selenite are lethal toxicants to plants and animals (Painter, 1941; Lemly, 2004). The outflow into environment at the high concentration causes serious adverse effects in the ecosystem. Therefore, selenium pollution and contamination of soil and aquatic habitats are worldwide problems and they are influenced to broad-range of human activities and environments. The allowable content and concentration in the environmental discharge is strictly regulated in various countries such as Japan, United States, Canada, United Kingdom and European Union. Accordingly, the further researches to explore the biological function which can apply to develop the recovery, removal and recycling technologies for selenium, should be undertaken.

A filamentous fungus (koji mold), *Aspergillus oryzae* has been widely used for fermentation, brewing and food production. In particular, this filamentous fungus is an essential microorganism for production of traditional Japanese foods such as sake (rice wine), miso (bean paste), shouyu (soy sauce) and so on (Machida, 2008). *A. oryzae* has served as a great contributor for development of Japanese cuisine from ancient times.

According to the recent molecular biology study of this fungus, the whole genome of *A. oryzae* strain RIB40 were completely sequenced and analysed in 2005 (Machida et al, 2005) . The report represents that the genome consists of eight chromosomes and 12074 genes (encoding proteins that consist of more than 100 amino acids) were included

in the about 37 mega base (Mb). The total genome size is larger 7-9 Mb than that of *A. nidulans* or *A. fumigatus*. A large number of enzymes that will be able to apply to effective conversion of organic compounds, is predicated and confirmed (Machida,2008, Machida et al, 2005). The existence of similar proteins (genes) for inorganic compounds can be easily expected.

Thus, in this study, we have investigated the possibility of selenium conversion and recovery by using *A. oryzae* strain RIB40 for its application into the elemental recycling and bioremediation of selenium.

MATERIALS AND METHODS

A. Effect of Selenite on Growth of A. oryzae on a Potato Dextrose Agar Plate

For investigation of growth on a potato dextrose agar (PDA) plate contained at various concentrations of selenite, conidia of *A. oryzae* strain RIB40 were formed on a PDA plate by the incubation at 30℃ for seven days. After gathering the formed conidia from the plate, the conidia were suspended in sterilized water (SDW), and the cell suspension (1.0 μl) was inoculated onto the centre of a fresh PDA plate contained sodium selenite at various concentrations among 0.5 mM to 2.0 mM. The plates were incubated at 30℃ for seven days. Diameter and colour of the colony of mycelium or conidium were measured and observed at third and seventh day of the incubation.

B. Cell Observation and Selenium Detection in Potato Dextrose Liquid Medium Contained Selenite

By using the same manner described in the previous part, conidia of *A. oryzae* strain RIB40 was formed on a PDA plate. The conidia was collected and suspended in SDW at a concentration of 1.0 x10^8 cells/ml. The cell suspension was inoculated to 20 ml fresh potato dextrose liquid (PDL) medium at an initial cell concentration of 1.0 x 10^6 cells/ml. After incubation for 22 hours at 30℃ with shaking at 120 rpm in a 100 ml baffled Erlenmeyer flask, the sterilized solution of sodium selenite was added into the culture at a final concentration of 0.5 mM, 1.0 mM, 2.0 mM, 3.0 mM and 5.0 mM, respectively. Incubation of the mycelial culture was continued in same conditions aerobically after selenite addition. Changes of colour and appearance of these cultures that contained sodium selenite at various concentrations (0 mM - 2.0 mM), were observed. Selenite concentration in the culture was measured by using an ion chromatography (DX-100, Dionex) with an analytical column for anion detection (IonPac, AS12A: 4 mm, Dionex) and the eluent (2.7 mM Na_2CO_3 and 0.3 mM $NaHCO_3$ solution). Experiments in this part were performed in triplicate or more.

C. Selenite Reduction and Recovery by the Granular Cell-Aggregates of A. oryzae

To investigate the growth inhibition of selenite, dry weight of the mycelia grown in a PDL medium contained selenite was measured by drying at 105˚C for two hours or overnight (about 6 hours) drying at 50˚C.

For confirmation of selenium recovery by the red-orange coloured granular cell-aggregates, the mycelia which were incubated in the presence of 1mM selenite was collected by filtration method (with a rayon polymer cloth, pore size: 22-25 μm, Miracloth, Merck Biosciences). After washing the collected cell-granules with SDW, 50 mg the dried cells was ground with a mortar. Powdered mold was completely lysed by using 3.0 ml of 1% (wt./vol.) NaOH and 1% (wt./vol.) hypochlorite solution, and then the lysate was diluted up to 25 ml by SDW with a volumetric flash. This solution was employed as analyte for atomic absorption spectroscopy (AA-6300, Shimazu) of selenium.

Moreover, for investigation of the selenium nanosphere formation, the transmission electron microscopic (TEM; JEM-2010, Jasco) observation and the elemental analysis (EDS; JED-2300T, Jasco) were carried out.

RESULTS AND DISCUSSIONS

Selenium Reduction and Growth Inhibition of A. oryzae Grown on a PDA Plate Contained Sodium Selenite

Changes of colour and appearance of *A. oryzae* strain RIB40 on a PDA plate which contained sodium selenite at various concentrations (0 mM -2.0 mM) were observed and compared (Fig. 1).

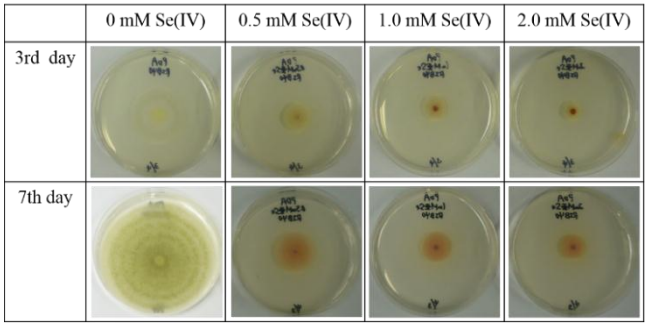

Fig. 1 Appearance of *A. oryzae* RIB40 grown on a PDA plate which contained selenite at various concentrations

Table I Effect of selenite on growth of *A. oryzae* on a PDA plate

Concentration	0 mM Se (IV)	0.5 mM Se (IV)	1.0 mM Se (IV)	2.0 mM Se (IV)
Diameter at 3 days (cm)	3.70±0.09	1.58±0.24	1.25±0.27	1.09±0.33
Diameter at 7 days (cm)	8.00* (maximum value)	2.85±0.79	2.20±0.50	2.01±0.36

*corresponding to the internal diameter of a disposable plate used in this experiment. Each experiment was carried out in more than triplicate (six plates were employed).

Cells of strain RIB40 could form the typical conidia on a PDA plate in the absence of sodium selenite, whereas the colony which was consisted of the mycelia appeared on the plate, and the normal development of conidia could not be observed in the presence of selenite as shown in Fig.1. The growth was inhibited at a high concentration of selenite. Diameter of the mycelia which formed on the plate became smaller with increasing the selenite concentration (Table I). Centre of the colony turned red-orange by the reduction of

selenite to elemental selenium. These results showed that the formed mycelia were able to convert anionic selenium ion into the insoluble selenium, even though the growth of *A. oryzae* was attenuated by addition of selenite.

Cell Observation and Selenite Reduction of Granular Cell-Aggregates of A. oryzae

When the conidia was inoculated to a fresh PDL medium, *A. oryzae* formed the granular cell-aggregates that consisted of the mycelia by the incubation for 22 hours (Fig. 2 A).

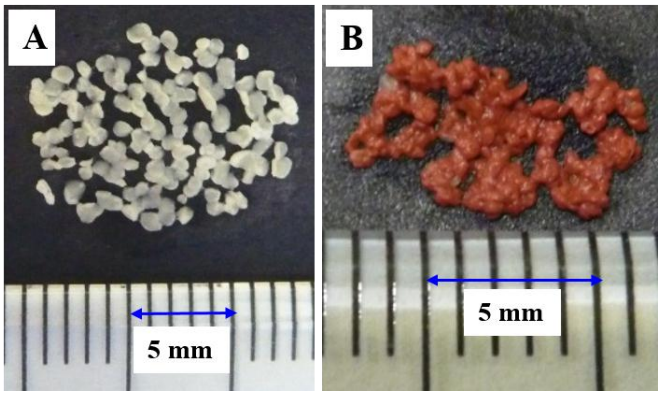

Fig. 2 Appearance of granular cell-aggregates of *A. oryzae* strain RIB40, which were incubated in a PDL medium aerobically.

A: Cell-aggregates that formed by the initial incubation for 22 hours in the absence of selenite.
B: Cell-aggregates contained red-orange colored selenium nanospheres that were formed by the further incubation of "A" for 72 hours after addition of sodium selenite (1 mM)

The cell-aggregates could well disperse in conditions shaken at 120 rpm in a PDL medium. In contrast, when the shaking was stopped, the cell-aggregate precipitated immediately on the bottom of the flask. Furthermore, cell elution to the medium solution and dissolution of the granular cell-aggregate have not been observed during the initial cultivation for formation of the cell-aggregates and its further incubation after addition of sodium selenite. The granular cell-aggregates were able to separate easily from the culture solution by a filtration without reducing pressure.

As shown in Fig. 2 B, cell-aggregates that formed by the initial incubation for 22 hours in a PDL medium, could be employed to reduce selenite into insoluble elemental selenium under aerobic condition. The selenium (IV) reduction was started under aerobic condition immediately after addition of selenite, and 1 mM selenite was completely disappeared from the PDL culture within 24 hours. These results represented that selenium could be recovered into the granular cell-aggregates without using complex procedures. Furthermore, it was possible to separate easily the granular cell-aggregates that contained the recovered selenium from the culture solution by a simple filtration procedure.

Selenium Recovery and Formation of Selenium Nanosphere

Selenium recovery rate at various initial selenite concentrations was summarized in Table II. When the initial selenite concentration was less than 1.0 mM, selenite in the culture after 72 hours were not detected. Selenium could be completely recovered from the PDL culture by cell-aggregates of *A. oryzae* strain RIB40. In addition, our further research revealed that selenite was completely disappeared from the PDL culture within 24 hours. The time for selenium recovery can be shortened. However, in the case of more than 2.0 mM, selenite remained in the culture. Complete recovery and conversion of selenite by cell-aggregates of *A. oryzae* strain RIB40 could be achieved when the initial selenite concentration was less than 1.0 mM. Moreover, TEM observation and EDS analysis of the cells and their electron-dense components indicated that selenite could be converted into selenium nanospheres aerobically.

Table II Selenium recovery rate at various initial selenite concentrations (72 hours incubation)

Initial selenite concentration	0.5 mM	1.0 mM	2.0 mM	3.0 mM	5.0 mM
Concentration of selenite in culture 72 hour (mM)	0±0	0±0	0.66±0.31	2.05±0.17	4.08±0.40
Recovery ratio of selenite (%)	100±0	100±0	65.97±15.13	36.23±4.31	22.56±2.62

CONCLUSIONS

In this study, we have investigated the possibility of selenium recovery and conversion by a filamentous fungus, *A. oryzae* strain RIB40. The granular cell-aggregates (mycelia) had the ability to reduce and convert selenite into selenium nanosphere when the granules were incubated under aerobic condition shaking at 120 rpm with a 100 ml baffled Erlenmeyer flask contained 20 ml PDL medium which included 1mM selenite. Furthermore, selenite was removed completely from the culture within 24 hours by the mycelial cell-granules. By using this technique, we have achieved to separate easily ionic selenium from the culture solution by a simple filtration without reducing pressure. These results demonstrate that *A. oryzae* strain RIB40 can be used as an effective bio-material for recovery and conversion of selenium-oxyanion in various industrial fields such as bioremediation, elemental resource recycling, and bio-manufacturing of selenium nanospheres.

ACKNOWLEDGMENT

We thank Dr. Maeda, the Natural Science Center for Basic Research and Development (N-BARD), Hiroshima University for the measurement of TEM-EDS. T.S. also thanks for the financial support to this research by Japan Society for the Promotion of Science (JSPS) of Grant-in-Aid for Scientific Research (C) (subject number: 24580482)

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