J. Microbiol. Biotechnol. (2002), **12**(2), 292–295

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Removal of Chromate by White Rot Fungus, *Inonotus cuticularis*

LEE, DONG-HEUB[1], YONG-WON MIN[2], HAE-IK RHEE[2], JAE E. YANG[3], GIE-TAEK CHUN[4], AND YEON-HO JEONG[2]*

[1]*Korea Forest Research Institute, Seoul 130-012, Korea*
[2]*Division of Biotechnology, Kangwon National University, Chunchon 200-701, Korea*
[3]*Division of Biological Environment, Kangwon National University, Chunchon 200-701, Korea*
[4]*Division of Life Sciences, Kangwon National University, Chunchon 200-701, Korea*

Received: November 10, 2001
Accepted: February 19, 2002

Abstract A chromate-resistant white rot fungus, *Inonotus cuticularis*, abundant in oak trees, was isolated for chromate removal and detoxification of chromate. *Inonotus cuticularis* was also investigated for an optimal waste treatment system. The screened cells were able to reduce an initial chromate concentration of as high as 1,300 ppm. Cell growth kinetics showed that the optimum culture conditions in flasks were at 33°C and pH 4.2. Furthermore, the cells were able to remove 54% of the initial chromate by a two-stage operation based on the combination of a fermentor and airlift reactor.

Key words: Chromate removal, *Inonotus cuticularis*, two-phase operation, fermentor, airlift reactor

The hexavalent chromium ion (chromate) is toxic and mutagenic for most organisms [8]. In humans, chromate causes irritation and corrosion of the skin and respiratory tract, and is believed to be responsible for lung carcinoma. Chromate is also hazardous to fauna and flora in natural aquatic ecosystems. Wastewater containing toxic chromate is generated in many industrial processes, including chrome leather tanning, chromium plating, metal cleaning and processing, wood preservation, and alloy preparation. Therefore, this wastewater must be treated before being discharged into natural environments. Several microorganisms that can reduce highly toxic chromate into less toxic trivalent chromium ion have already been suggested as possible agents for treating chromate-contaminated wastewater [1-4, 7-14, 16]. Most chromate-reducing microorganisms reported so far are bacteria, and most white rot fungi have been reported in relation to the biodegradation of organic compounds [5, 6]. However, the current study reports on a recently isolated chromate-resistant fungus, *Inonotus cuticularis*, as a possible chromate-reducing microorganism. During aerobic growth, the isolated white rot fungus can reduce chromate into the trivalent chromium ion (Cr^{+3}) which can be subsequently precipitated in the culture medium [12]. The cells are capable of reducing an initial concentration of chromate of as high as 1,300 ppm, which has never been achieved by other microorganisms reported so far. Based on these observations, the current study investigated the applicability of this white rot fungus to chromate removal. The optimum culture conditions and an operating strategy for the development of an efficient large-scale chromate removal system are suggested.

*Corresponding author
Phone: 82-33-250-6484; Fax: 82-33-254-3835;
E-mail: jeongyhb@cc.kangwon.ac.kr

Materials and Methods

Cells and Culture Condition

A chromate-resistant white rot fungus, *Inonotus cuticularis* (FRI 20621), abundant in oak trees, was screened by the Forestry Research Institute, Korea. The fungus was selected from colonies growing on the surface of woods treated with a preservative containing chromate, copper, and arsenate. To maintain the fungal cells on a solid culture, a solid medium containing agar 25 g/l, glucose 20 g/l, KH_2PO_4 3 g/l, $MgSO_4$ 2 g/l, peptone 5 g/l, and malt extract 10 g/l was used. Agar, glucose, KH_2PO_4, $MgSO_4$, and peptone were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and malt extract from Difco (Detroit, MI, U.S.A.). The cells were cultivated at 33°C in an incubator for 10 days and preserved in a refrigerator at 4°C for subculturing. For the liquid suspension culture, the mycelia of the fungus were grown in a broth medium at 33°C in a shaking incubator at 200 rpm. The broth medium had the same composition as the solid culture medium except for the agar. To determine the optimum culture conditions, cell

growth curves at various pHs and temperatures were compared by monitoring the change in the dry cell weight of the fungal cells in a 500 ml liquid culture using a Sagaguchi shaking flask.

Removal of Chromate Using Suspension Culture of *Inonotus cuticularis*

The chromate-removal performance was investigated using a single-stage operation in which chromate was removed simultaneously with the growth of fungal cells in a single vessel. *Inonotus cuticularis* cells were inoculated into broth media containing an initial chromate concentration of 1,300 ppm, and cultured at 33°C and pH 4.2 at 200 rpm for 5 days. The cells were harvested by filtration and washed three times with phosphate buffer. The change in the dry cell weight of the fungal cells was monitored and the total chromate concentrations were assayed by Shimadzu EM 109 atomic absorption spectrophotometry (Shimadzu, Tokyo, Japan). A two-stage operation was tested to overcome the disadvantages of a single-stage operation. For the two-stage operation, cells were produced by employing a 2.5-l fermentor system (Kobiotech Co., Ltd., Inchon, Korea) in the first stage, and in the second stage, wastewater containing chromate was treated using the cells from the first stage. A fermentor-fermentor (800 ml), fermentor-shaker (800 ml), and fermentor-airlift reactor (800 ml) were all incorporated together for the two-stage operation.

Results and Discussion

It is desirable to operate the treating vessel under optimum growth conditions so that the degrading microorganism can compete with other microorganisms when chromate is treated with the degrading cells in the same vessel (one-stage operation). In a two-stage operation, it is also desirable to rapidly propagate the degrading cells in the first stage. As such, it is important to establish optimum growth conditions for the degrading microorganism before developing a wastewater treatment system. Figure 1 shows the effect of pH on the growth of *Inonotus cuticularis* at 28°C, showing optimum pH of 4.2 for cell growth. Figure 2 shows the effect of temperature on the growth of *Inonotus cuticularis* at pH 4.2. The cell growth rate increased with

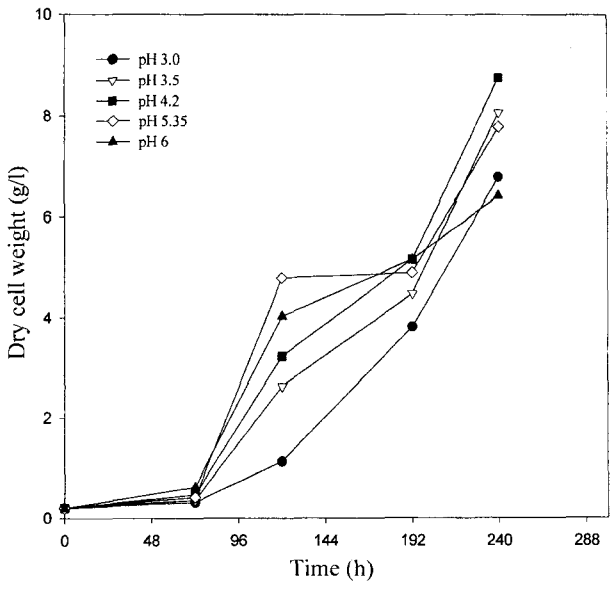

Fig. 1. Effect of pH on cell growth of *Inonotus cuticularis*.

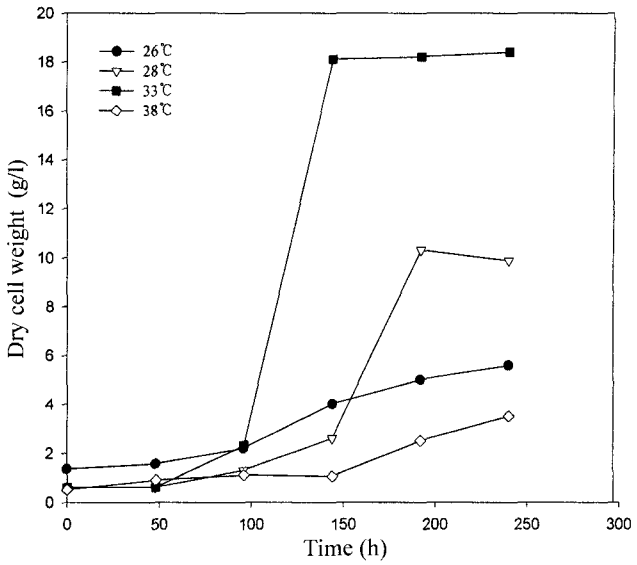

Fig. 2. Effect of temperature on cell growth of *Inonotus cuticularis*.

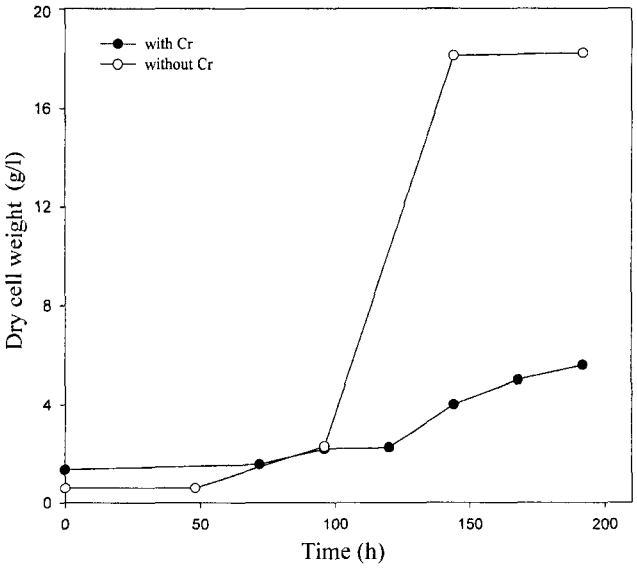

Fig. 3. Effect of chromium concentration on cell growth of *Inonotus cuticularis*.

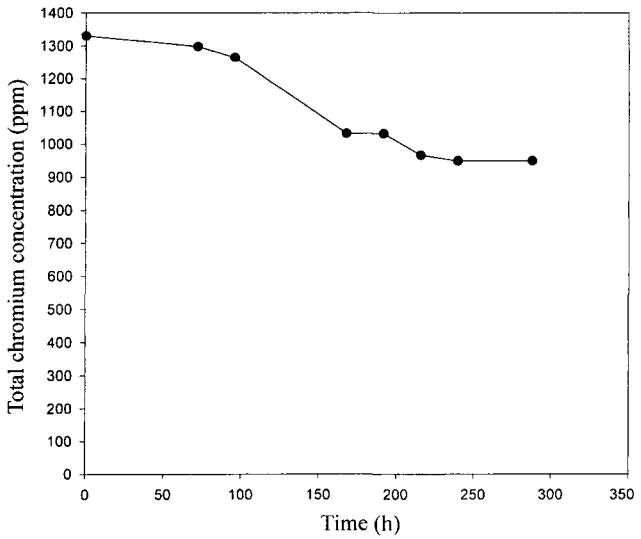

Fig. 4. Removal kinetics of total chromium by *Inonotus cuticularis* in shake flask (single-stage operation, C_i=1,300 ppm).

the temperature up to 33°C, however, the growth rate declined at temperatures higher than 33°C. Therefore, the optimum temperature for cell growth was determined to be 33°C.

Figures 3 and 4 show the results of the single-stage operation, in which the cells were inoculated into media containing 1,300 ppm of chromate and cultured in a shake flask. Figure 3 shows that considerable cell growth occurred even in a 1,300 ppm chromate solution. This indicated that the screened white rot fungus was resistant to high concentration of chromate, however, the cell growth rate was retarded by the presence of chromate. Therefore, it is expected that growing cells and treating chromate in the same vessel (single-stage operation) would be inefficient.

Figure 4 shows the change in the total residual concentration of chromium ion relative to time. It was quite noticeable that the screened fungal cells were able to remove an initial concentration of chromate as high as 1,300 ppm. As of now, the bacterial reduction of chromate has been widely reported, however, there have been few reports on chromate removal by fungal cells. Accordingly, the current results provide evidence that not only bacterial cells but also fungal cells can reduce toxic chromate to the less toxic trivalent chromium ion. Hopefully, this may stimulate research activity on the screening of more powerful fungal cells for chromate reduction. Furthermore, the current results showed that the screened cells had the ability of reducing an initial concentration of chromate as high as 1,300 ppm, which had never been achieved by any other microorganism reported so far. Chromate reduction by *Enterobacter cloacae* in an initial chromate concentration of 26 ppm [15], by *Pseudomonas putida* in 1.6 ppm [7], by *Pseudomonas ambigua* in 10 ppm [13], by *Agrobacterium radiobacter* in 26 ppm [9], and by *Desulfovibrio vulgaris* in 26 ppm [10] has previously been reported. Therefore, the *Inonotus cuticularis* cells in the current work demonstrated that they could be used as a degrading microorganism, especially for treating wastewater containing a high concentration of chromate. However, the figure also shows that only about 30% of the initial chromate was removed, indicating inefficiency of the single-stage operation, as expected. Thus, in order to improve the single-stage operation to perform at their maximum capability, a two-stage operation was suggested, in which the cells were rapidly propagated under chromate-free conditions in the first stage and then brought into contact with chromate-containing wastewater in the second stage.

Figure 5 shows the result of the two-stage operation. The cells were cultivated in a fermentor in the first stage and then used to treat a chromate solution of about 900 ppm in the second stage. For comparison, a shake flask and fermentor were both employed as the second stage. According to the figure, the chromate removal was about 52% when the fermentor was used in the second stage, which was higher than that when the shaker was used (33%). This might have been due to advantages of a fermentor over a shaker such as easy maintenance of the optimum conditions and fast mass transfer.

Figure 6 shows chromate removal kinetics in the two-stage operation when the combination of a fermentor and airlift reactor was used. The use of a fermentor for cell growth is more desirable because of the enhanced oxygen transfer due to mechanical agitation; however, a new type of treating vessel for the second stage is still required to reduce high energy cost and cell damage by stirring, because the mixing with the wastewater is more important than cell growth in the second stage. Consequently, an

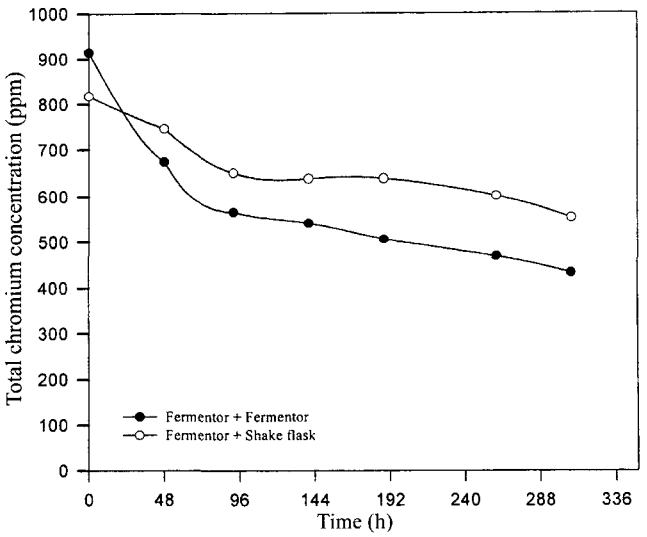

Fig. 5. Comparison of removal kinetics of total chromium by *Inonotus cuticularis* between fermentor and shake flask (two-stage operation).

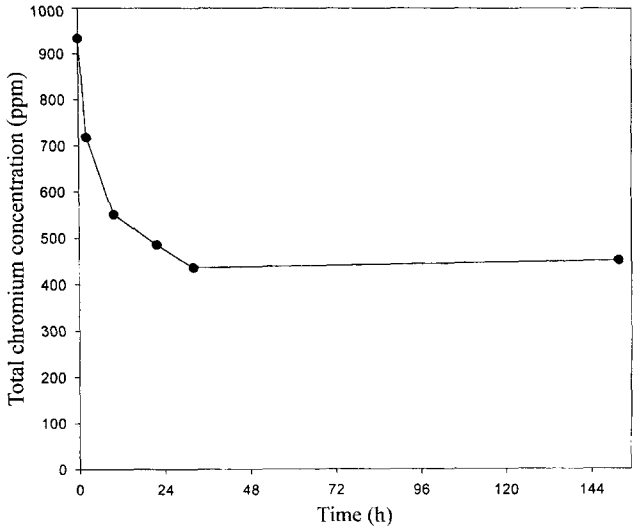

Fig. 6. Removal kinetics of total chromium by *Inonotus cuticularis* in integrated system of fermentor and airlift reactor (two-stage operation).

airlift reactor was suggested as the second-stage treating vessel. In an airlift reactor, the mixing occurs by fluid circulation, because of the density difference between the air bubble rising zone and the stagnant zone. The results showed that about 54% of the initial chromate was removed by the two-stage operation when the combination of a fermentor and airlift reactor was used.

CONCLUSIONS

A chromate-resistant white rot fungus, *Inonotus cuticularis*, abundant in oak trees, was isolated for chromate removal and detoxification, and an optimal waste treatment system employing the fungus was investigated. The optimum cell growth conditions for the chromium-resistant white rot fungus were found to be 33°C and pH 4.2. The screened cells exhibited the ability similar to bacterial cells to reduce toxic chromate into less toxic trivalent chromium ion. Furthermore, the screened fungal cells were able to remove an initial concentration of chromate as high as 1,300 ppm, which has never been achieved before by any other microorganism. The percentage of total chromium ion removal was about 30% when the cells were in contact with chromate from the beginning in a flask, indicating that the cell growth was inhibited by the presence of chromate. Therefore, a two-stage operation was attempted using a stirred reactor and airlift reactor system, and about 54% total chromium ion removal was attained. Accordingly, the current results suggest industrial-scale use of *Inonotus cuticularis* cells in an appropriate reactor system, such as a two-stage reactor system with a stirred reactor and airlift reactor, to remove chromate from wastewater.

REFERENCES

1. Bae, W. C., T. G. Kang, I. K. Kang, Y. J. Won, and B. C. Jeong. 2000. Reduction of hexavalent chromium by *Escherichia coli* ATCC33456 in batch and continuous cultures. *J. Microbiol.* **38:** 36–39.
2. Bae, W. C., T. G. Kang, J. H. Jung, C. J. Park, S. C. Choi, and B. C. Jeong. 2000. Purification and characterization of NADH-dependent Cr(VI) reductase from *Escherichia coli* ATCC33456. *J. Microbiol. Biotechnol.* **10:** 580–586.
3. Chirwa, E. M. N. and Y. T. Wang. 1997. Chromium(VI) reduction by *Peudomonas fluorescens* LB300 in fixed film bioreactor. *J. Environ. Eng.* **123:** 760–766.
4. Chirwa, E. M. N. and Y. T. Wang. 1997. Hexavalent chromium reduction by *Bacillus* sp. in packed bed bioreactor. *Environ. Sci. Technol.* **31:** 1446–1451.
5. Choi, S. H., S. H. Moon, J. S. Lee, and M. B. Gu. 2000. Biodegradation of 2,4,5-trichlorophenol using cell-free culture broths of *Phanerochaete chrysosporium*. *J. Microbiol. Biotechnol.* **10:** 759–763.
6. Hwang, S. S. and H. G. Song. 2000. Biodegradation of pyrene by the white rot fungus, *Irpex lacteus*. *J. Microbiol. Biotechnol.* **10:** 344–348.
7. Ishibashi Y., C. Cervantes, and S. Silver. 1990. Chromium Reduction in *Peudomonas putida*. *Appl. Environ. Microbiol.* **56:** 2268–2270.
8. Komori, K., A. Rivas, K. Toda, and H. Ohtake. 1990. Biological removal of toxic chromium using an *Enterobacter cloacae* strain that reduces chromate under anaerobic condition. *Biotechnol. Bioeng.* **35:** 951–954.
9. Llovera, S., R. Bonet, M. D. Simon-Pujol, and F. Congregado. 1993. Chromate reduction by resting cells of *Agrobacterium radiobacter* ESP-916. *Appl. Environ. Microbiol.* **59:** 3516–3518.
10. Lovley, D. R. and E. J. P. Phillips. 1994. Reduction of chromate by *Desulfovibrio vulgaris* and its c_3 cytochrome. *Appl. Environ. Microbiol.* **60:** 726–728.
11. Shen, H. and Y. T. Wang. 1993. Modeling hexavalent chromium reduction in *Escherichia coli* 33456. *Biotechnol. Bioeng.* **43:** 293–300.
12. Stahl, J. D., S. J. Rasmussen, and S. D. Aust. 1995. Reduction of quinones and radicals by a plasma membrane redox system of *Phanerochaete chrysosporium*. *Arch. Biochem. Biophy.* **322:** 221–227.
13. Suzuki, T., N. Miyata, H. Horitsu, K. Kawai, K. Takamizawa, Y. Tai, and M. Okazaki. 1992. NAD(P)H-dependent chromium (VI) reductase of *Pseudomonas ambigua* G-1: A Cr(V) intermediate is formed during the reduction of Cr(VI) to Cr(III). *J. Bacteriol.* **174:** 5340–5345.
14. Wang, P. C., T. Mori, K. Komori, M. Sasatsu, K. Toda, and H. Ohtake. 1989. Isolation and characterization of an *Enterobacter cloacae* strain that reduces hexavalent chromium under anaerobic conditions. *Appl. Environ. Microbiol.* **55:** 1665–1669.
15. Wang, P. C., T. Mori, K. Toda, and H. Ohtake. 1990. Membrane-associated chromate reductase activity from *Enterobacter cloacae*. *J. Bacteriol.* **172:** 1670–1672.
16. Williams, J. W. 1984. Bacterial resistance and detoxification of heavy metals. *Enzyme. Microb. Technol.* **6:** 530–537.