JES
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
2013 Volume 25 Supplement
www.jesc.ac.cn

The 5th International Symposium on Environmental Economy and Technology

ELSEVIER
Science Press

Sponsored by
Research Center for Eco-Environmental Sciences
Chinese Academy of Sciences

ISSN 1001–0742 **Journal of Environmental Sciences** Supplement 2013

CONTENTS

Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2013, 25(Suppl.) S164–S168
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Decolorization of oxygen-delignified bleaching effluent and biobleaching of oxygen-delignified kraft pulp by non-white-rot fungus *Geotrichum candidum* Dec 1

Noboru Shintani[1,2,*], Makoto Shoda[2]

1. Advanced Science Research Laboratory, Saitama Institute of Technology, 1690 Fusaiji, Fukaya-city, Saitama 3690293, Japan
2. Chemical Resources Laboratory, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 2268503, Japan

Abstract

Decolorization of oxygen-delignified bleaching effluent (abbreviated as OBE) and biobleaching of oxygen-delignified kraft pulp (OKP) were conducted using a non-white rot fungus *Geotrichum candidum* Dec 1 (abbreviated as Dec 1) which has ability to decolorize various synthetic dyes and molasses. Dec 1 decolorized up to 77% of OBE for 6 days. In addition, Dec 1 increased the brightness of OKP from 47.8% to 51.2% and decreased the kappa value of OKP from 12.4 to 10.4 points during a 6-day incubation period at a 25% of pulp-concentration. At 2% pulp-concentration, the brightness of OKP increased by 13% and the kappa value of OKP decreased by 4 points only for a 3-day incubation period. When the decolorized OBE was used for bleaching of OKP, the brightness of OKP increased to 62.7% under the shaking culture to a 2% pulp-concentration using culture fluid of decolorized OBE. It was revealed that Dec 1 is a potential to apply for decolorization of wastewater and biobleaching of pulp in paper-mills.

Key words: decolorization; oxygen-delignified bleachning effluent; biobleaching; oxygen-delignified kraft pulp; non-white rot fungus *Geotrichum candidum* Dec 1

Introduction

It is difficult to remove the existing lignin in unbleached kraft pulp (UKP). Therefore, the multistage bleaching process with the chlorine-based chemical is used. However, the chlorine-based multistage bleaching process causes environmental pollution. This is because much environmental pollutant (such as dioxin) is included in effluent drained from chlorine-based multistage bleaching process (Kringstad et al., 1984; Smeds et al., 1994).

Therefore, the development of different bleaching process to reduce the consumption of the chlorine-based chemical is necessary to solve this problem. One of this method is oxygen bleaching process, and the de-lignin of pulp by oxygen bleaching process becomes the important bleaching process (Maria et al., 1997). However, it is necessary to remove colored substance (mainly lignin) in oxygen-delignified bleaching effluent (OBE) drained from oxygen bleaching process, because much colored substance is included in OBE and approximately 30% of this colored substance is carried over to the next bleaching process.

There is the biobleaching process to remove the lignin of pulp with the microbe and the enzyme as other methods. The content of residual lignin of oxygen-delignified kraft pulp (OKP) after oxygen-delignified bleaching is half of UKP and this is suitable for the bleaching due to the microbe (Reid and Paice, 1994). Therefore, biobleaching of OKP using white rot fungus such as *Trametes versicolor* (Reid and Paice, 1994), IZU-154 (Murata et al., 1992), *Bjerkandera* sp. strain BOS55 (Moreira and Feijoo, 1997), SKB-1152 (Iimori et al., 1994) is studied. However, no study of biobleaching of OKP using the non white-rot fungi has been reported.

We screened a novel decolorizing fungus, *Geotrichum candidum* Dec 1 (Dec 1), which has isolated from the soil, and Dec1 is on-white-rot fungus (McMullan et al., 2001). Dec1 has decolorized 30 synthetic dyes, molasses (Kim et al., 1995) and kraft pulp bleaching effluent (Shintani et al., 2002). Dec1 secretes enzyme having a unique and novel per-oxidase as well as manganese peroxidise (Kim and Shoda, 1999). Therefore, we applied a non-white rot fungus Dec1 to the decolorization of OBE and to the bleaching of OKP.

* Corresponding author. E-mail: nshintani@sit.ac.jp

1 Materials and methods

1.1 Strain

We used Non-white-rot fungus *Geotrichum candidum* Dec 1 (Dec 1), which decolorized 30 kinds of synthetic dyes and showed tolerance in highly-concentrated dyes (Kim et al., 1995)

The suspension of Dec 1 spores was stocked in 25% glycerol at –80°C.

1.2 OBE and oxygen-delignified kraft pulp (OKP)

The effluent at the oxygen extraction stage of hard wood pulp provided by Oji Paper Co. was used as the oxygen-delignified bleaching effluent (OBE).

The pulp after the oxygen extraction stage of hard wood pulp provided by Oji Paper Co. was used as the oxygen-delignified kraft pulp (OKP). The initial brightness and kappa value of OKP were 47.8% and 12.4, respectively.

1.3 Pre-culture of Dec1

Potato dextrose (PD) medium was prepared by the following method. Commercial potato (100 g) was cut to blocks around 1 cm^3 and boiled in 1 L of water for 30 min. Then, the potato was mashed and the suspension was filtered using filter paper and vacuum aspiration. Glucose (20 g) was added to the obtained filtrate. This solution was made up to 1 L with distilled water and adjusted to pH 5.5. A 10-μL aliquot of 25% glycerol stock suspension of Dec1 spore was inoculated on a PDA agar plate and incubated for 10 days at 30°C. The mycelia on four plates were collected and suspended in 10 mL of sterilized water with a vortex mixer. The suspension was filtered with gauze, and the filtrate was obtained as a fresh spore suspension.

1.4 Decolorization of OBE by Dec1

1.4.1 Decolorization

The OBE was adjusted to pH 5.5 with 2 mol/L HCl and centrifuged to remove insoluble particles (6000 r/min for 60 min). Glucose (1.5 or 4.5 g), ammonium tartrate (0.075 g), KH_2PO_4 (0.15 g), $MgSO_4{\cdot}7H_2O$ (0.075 g), and yeast extract (0.45 g) were added to 150 mL of OBE in 500 mL shake flask and sterilized (120°C, 20 min). These media were named as OGY 10 medium and OGY30 medium, depending on glucose concentration.

Dec1 spore suspension (5 mL) was inoculated into the flask and the flask was shaken with a reciprocal shaker (120 r/min) at 30°C for 7 days.

1.4.2 Color removal

The color of the effluent was determined by the standard method of the Canadian Pulp and Paper Association (Nagarathnamma and Pratima, 1999). The absorbance at 465 nm of cell-free solution against distilled water was measured using a spectrophotometer (UV-2200, Shimadzu, Japan). The absorbance values were then transformed into color units (CU) according to the following formula.

$$CU = 500 \times A_2 / A_1$$

where, A_1 corresponds to the A_{465} of a 500-CU platinum-cobalt standard solution (0.132) and A_2 is the absorbance of the cell-free effluent sample. The color removal (%) was defined as the ratio of CU of the cell-free culture supernatant to that of the initial medium.

1.5 Biobleaching of OKP by Dec1 grown in DGY30 medium

1.5.1 Cultivation of Dec1

Glucose (4.5 g), ammonium tartrate (0.075 g), KH_2PO_4 (0.15 g), $MgSO_4{\cdot}7H_2O$ (0.075 g), and yeast extract (0.45 g) (named DGY30 medium) were added to 150 mL of the distilled water in 500 mL shake flask and sterilized (120°C, 20 min). Dec1 spore suspension (5 mL) was inoculated into the flask and the flask was shaken with a reciprocal shaker (120 spm) at 30°C for 3 days.

1.5.2 Biobleaching of OKP by Dec1 cultured in DGY30 medium

A 4-g of OKP in 180 mL of the distilled water (2% (*w*/*V*) of pulp concentration) in 500 mL shake flask was sterilized (120°C, 20 min) and 20 mL of culture including Dec1 grown in DGY30 medium for 3 days was added in the shake flask, then the flask was shaken with a reciprocal shaker (120 r/min) at 30°C for 15 days (Iimori et al., 1994).

On the other hand, 25% of pulp-concentration was prepared and biobleached under the similar condition as 2% pulp concentration at 30°C for 12 days.

1.6 Biobleaching of OKP using the culture fluid after the decolorization of OBE by Dec1

1.6.1 Culture after the decolorization of OBE by Dec1

The OBE decolorized by Dec1 in OGY30 medium at 30°C for 3 days was prepared.

1.6.2 Biobleaching of OKP with the culture filtrate

The OBE prepared above was filtered through the gauze and then filtered by a membrane filter (0.45 μm). The 20 mL of filtrate was added in 500 mL shake flask including 80 mL of sterilized water and 2 g of OKP (2% of pulp-concentration) and shaken with a reciprocal shaker (120 r/min) at 30°C for 7 days. As a control, the 500 mL shake flask including 200 mL of sterilization water and 4 g of OKP were tested.

1.7 Measurement of the brightness and the kappa value of pulp

After the biobleaching of OKP, OKP was sterilized (120°C, 20 min), washed with distilled water. A sheet of OKP prepared was prepared by washed OKP, and the brightness of a sheet of OKP prepared was determined with a Spectrophotometric colorimeter (SUGA Test Instruments Co., Ltd., Japan) according to JIS (Japanese Industrial

Standard) P 8209 and JIS P 8123. The Kappa value of the sheet was measured according to TAPPI standard (Katagiri et al., 1995).

1.8 Enzyme assay

1.8.1 DyP assay

DyP is a novel peroxidise produced by *G. candidum* Dec1 which was associated with decolorization of dyes (Kim and Shoda, 1999). Reactive Blue 5 (RB5; ε_{600} = 8800 $M^{-1}cm^{-1}$), a representative anthraquinone dye, was used as the substrate of DyP. The analytical procedure was described in a previous paper and one unit of enzyme activity was defined as the amount of the enzyme that decolorized 1 μmol of RB5 at 30°C for 1 min (Shintani et al., 2002).

1.8.2 Manganese peroxidase assay

Guaiacol was used as the substrate for the manganese peroxidise (MnP) assay. The analytical procedure was described in a previous paper and one unit of enzyme activity was defined as the amount of the enzyme that reacted with 1 μmol of guaiacol at 30°C for 1 min (Shintani et al., 2002).

1.8.3 Lignin peroxidase assay

Veratryl alcohol was used as the substrate for the lignin peroxidise (LiP) assay. The analytical procedure was described in a previous paper and one unit of enzyme activity was defined as the amount of the enzyme that reacted with 1 μmol of veratryl alcohol at 30°C for 1 min (Shintani et al., 2002).

1.8.4 Laccase assay

2,2′-Azino-di-(3-ethylbenzthiazoline)-6′-sulfonate (ABTS) was used as the substrate for the laccase (lac) assay. The analytical procedure was described in a previous paper and one unit of enzyme activity was defined as the amount of the enzyme that reacted with 1μmol of ABTS at 30°C for 1 min (Shintani et al., 2002).

2 Results and discussion

2.1 Decolorization of OBE by Dec1

The time courses for color removal and the pH of OBE at two glucose concentrations are shown in **Fig. 1**. Color removal was 65% after 3 days of culture in OGY 10 medium, but the pH increased when color removal decreased after 4 days. Because this has a lower glucose concentration, consumption of glucose disappears, and it is thought that the metabolism by Dec1 stopped.

The color removal was 72% after 3 days of culture and levelled off in OGY30 medium. In the medium of 0 g/L of glucose, the color removal was 0% (data not shown).

The time courses of color removal and enzyme activities of the culture supernatant in OGY30 medium are shown

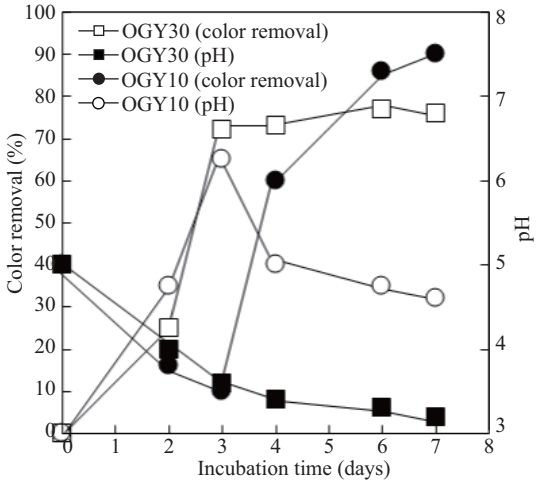

Fig. 1 Time courses for color removal and the pH of OBE at two glucose concentrations.

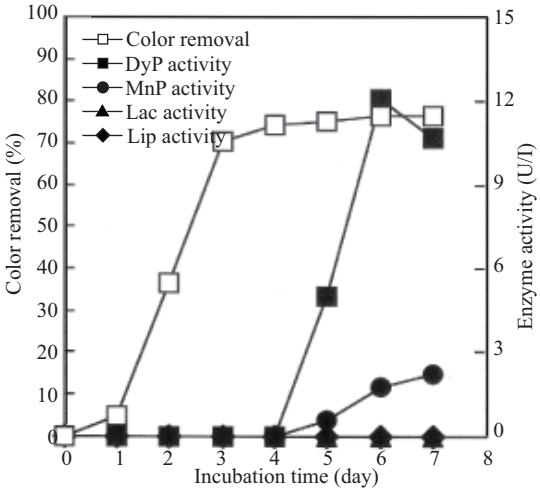

Fig. 2 Time courses for color removal and enzyme activities of OBE in OGY30 medium.

in **Fig. 2**. Color removal increased similarly to **Fig. 1**. DyP and MnP activities appeared in the culture, but the activities of LiP and Lac were not observed.

Although the mechanism is unknown, color removal by Dec1 is thought to proceed through two steps. The first step is color adsorption to the cells. Actually, the color of the cell pellet was observed to be enhanced gradually up to 3 to 4 days of culture. The second step is decomposition of the adsorbed colored substance by enzyme. After 4 days, enzyme activity is seen, and it is thought that the adsorbed colored substance is decomposed by enzyme.

2.2 Biobleaching of OKP by Dec1 cultured in DGY30 medium

The time courses of the biobleaching of OKP (2% pulp-concentration) by Dec1 cultured in DGY30 medium are shown in **Fig. 3**. Dec 1 increased the brightness of OKP from 47.8% to 60.3% and decreased the kappa value of OKP from 12.4 to 8.1 points during a 3-day incubation period of a 2% of pulp-concentration.

In the case of 25% pulp-concentration of OKP, the brightness of OKP increased only by approximately 4% (52.1% from 47.8%) and the kappa value of OKP decreased by 3 points during a 12-day incubation period (data not shown). It was considered that oxygen is essential in the decolorization of various synthetic dyes by Dec1 (Kim et al., 1995), oxygen supply may be limiting by high pulp-concentration.

The time courses of enzyme activities during OKP (2% pulp-concentration) bleaching are shown in **Fig. 4**. During bleaching of OKP, DyP and MnP activities appeared but the activities of LiP and Lac were not observed.

2.3 Biobleaching of OKP using the culture fluid after the decolorization of OBE by Dec 1

In **Fig. 1**, Dec1 decolorized 70% of OBE for 3 days. Therefore, by using the culture after decolorization of OBE, we attempted to bleach OKP (2% pulp-concentration). As shown in **Fig. 5**, the brightness of OKP increased to 62.7% under the shaking culture of a 2% pulp-concentration during a 7-day incubation period. In the case of a cell-free culture, the brightness of OKP increased by 4 points during a 3-day incubation period, but the increasing the brightness of OKP was not seen after 3 days (data not shown).

The time courses of enzyme activities during OKP (2% pulp-concentration) bleaching using the culture fluid after the decolorization of OBE by Dec1 are shown in **Fig. 6**. DyP and MnP activities appeared during OKP biobleaching. On the other hand, the activities of LiP and Lac were not observed.

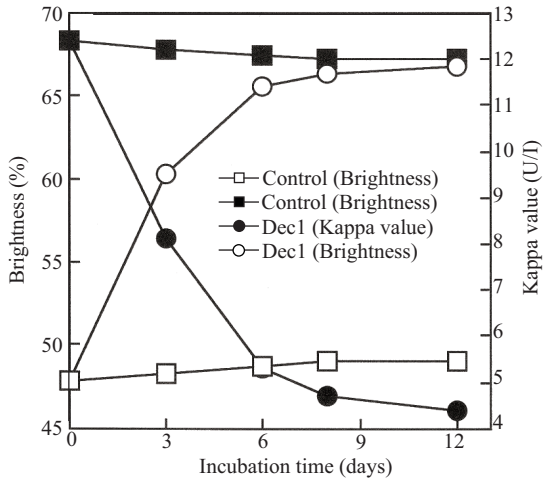

Fig. 3 Time courses of the biobleaching of OKP (2% pulp-concentration) by Dec 1 grown in DGY30 medium.

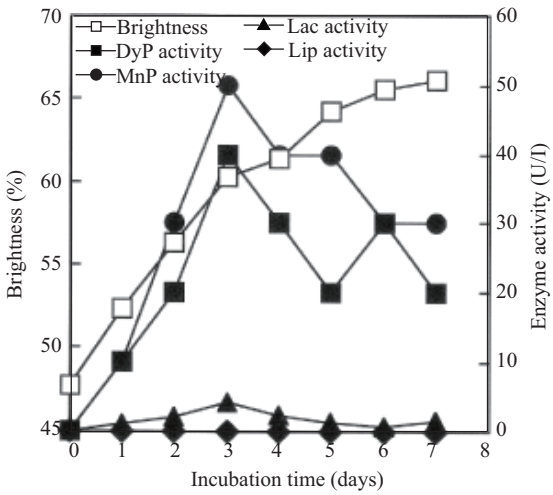

Fig. 4 Time courses of enzyme activities during OKP (2% pulp-concentration) bleaching by Dec 1 grown in DGY30 medium.

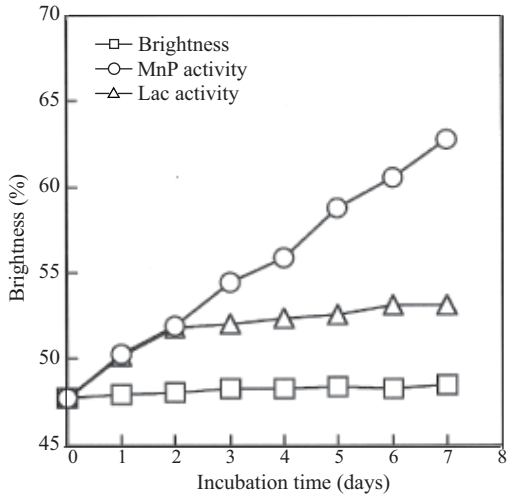

Fig. 5 Time courses of the biobleaching of OKP (2% pulp-concentration) using the cell-free culture and the culture fluid after the decolorization of OBE by Dec 1.

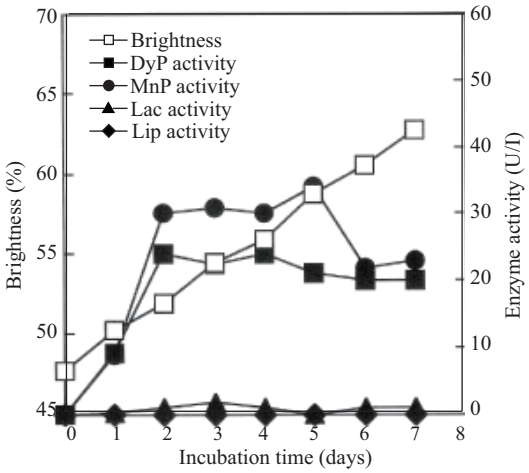

Fig. 6 Time courses of enzyme activities during OKP (2% pulp-concentration) biobleaching using the culture fluid after the decolorization of OBE by Dec 1.

3 Conclusions

Dec 1 decolorized up to 77% of OBE for 6 days. The brightness of OKP (2% pulp-concentration) by Dec 1

cultured in DGY medium increased from 47.8% to 60.3% and Kappa number decreased from 12.4 to 8.1 points during 3-day incubation period. Furthermore, using the culture fluid after the decolorization of OBE by Dec 1, the brightness of OKP (2% pulp-concentration) increased from 47.8% to 62.7% during 7-day incubation period.

These results clearly showed that Dec 1 had ability to decolorize OBE and to bleach oxygen-delignified Kraft pulp.

References

Iimori T, Kaneko R, Yoshikawa H, Machida M, Yoshioka H, Murakami K, 1994. Screening of pulp-bleaching fungi and bleaching activity of newly isolated fungus SKB-1152. *Mokuzai Gakkaishi*, 40(7): 733–737.

Katagiri N, Tsutsumi Y, Nishida T, 1995. Correlation of brightening with cumulative enzyme activity related to lignin biodegradation during biobleaching of kraft pulp by white rot fungi in the solid-state fermentation system. *Applied and Environmental Microbiology*, 61(2): 617–622.

Kim S J, Ishikawa K, Hirai M, Shoda M, 1995. Characteristics of a newly isolated fungus, *Geotrichum candidum* Dec 1. *Journal of Fermentation and Bioengineering*, 79(6): 601–607.

Kim S J, Shoda M, 1999. Decolorization of molasses and a dye by a newly isolated strain of the fungus *Geotrichum candidum* Dec 1. *Biotechnology and Bioengineering*, 62(1): 114–119.

Kringstad K P, Lindstrom K, 1984. Spent liquors from pulp bleaching. *Environmental Sciences and Technology*, 18(18): 236A–248A.

Maria T M, Gumersindo F, Reyes S, Juan L, Jim A F, 1997. Biobleaching of oxygen delignified kraft pulp by several white rot fungal strains. *Journal of Biotechnology*, 53: 237–251.

McMullan G, Meehan C, Conneely A, Kirby N, Robinson T, Nigam Pet al., 2001. Microbial decolourisation and degradation of textile dyes. *Applied Microbiology and Biotechnology*, 56(11): 81–87.

Moreira M T, Feijoo G, Sierra-Alvarez R, Lema J, Field J A, 1997. Manganese is not required for biobleaching of oxygen-delignified kraft pulp by the white rot fungus *Bjerkandera* sp. strain BOS55. *Applied and Environmental Microbiology*, 63(5): 1749–1755.

Murata S, Kondo R, Sakai K, Kashino Y, Nishida T, Takahara Y, 1992. Cholorine free bleaching process of kraft pulp using treatment with the fungus IZU-154. *Tappi Journals*, 75(12): 91–94.

Nagarathnamma R, Pratima B, 1999. Decolorization and detoxification of extraction stage effluent from chlorine bleaching of kraft pulp by *Rhizopus oryzae*. *Applied and Environmental Microbiology*, 65(3): 1078–1082.

Reid I A, Paice M G, 1994. Effect of residual lignin type and amount on bleaching of kraft pulp by *Trametes versicolor*. *Applied and Environmental Microbiology*, 60(5): 1395–1400.

Shintani N, Sugano Y, Shoda M, 2002. Decolorization of kraft pulp bleaching effluent by a newly isolated fungus, *Geotrichum candidum* Dec 1. *Journal of Wood Science*, 48:402–408.

Smeds A, Holmbom B, Pettersson C, 1994. Chemical stability of chlorinated component in pulp bleaching liquors. *Chemosphere*, 28(28): 881–895.

Editorial Board of Journal of Environmental Sciences

JOURNAL OF ENVIRONMENTAL SCIENCES

环境科学学报(英文版)

(http://www.jesc.ac.cn)

Aims and scope

Journal of Environmental Sciences is an international academic journal supervised by Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. The journal publishes original, peer-reviewed innovative research and valuable findings in environmental sciences. The types of articles published are research article, critical review, rapid communications, and special issues.

The scope of the journal embraces the treatment processes for natural groundwater, municipal, agricultural and industrial water and wastewaters; physical and chemical methods for limitation of pollutants emission into the atmospheric environment; chemical and biological and phytoremediation of contaminated soil; fate and transport of pollutants in environments; toxicological effects of terrorist chemical release on the natural environment and human health; development of environmental catalysts and materials.

For subscription to electronic edition

Elsevier is responsible for subscription of the journal. Please subscribe to the journal via http://www.elsevier.com/locate/jes.

For subscription to print edition

China: Please contact the customer service, Science Press, 16 Donghuangchenggen North Street, Beijing 100717, China. Tel: +86-10-64017032; E-mail: journal@mail.sciencep.com, or the local post office throughout China (domestic postcode: 2-580).

Outside China: Please order the journal from the Elsevier Customer Service Department at the Regional Sales Office nearest you.

Submission declaration

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere. The submission should be approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out. If the manuscript accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Submission declaration

Submission of the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere. The publication should be approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out. If the manuscript accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Editorial

Authors should submit manuscript online at http://www.jesc.ac.cn. In case of queries, please contact editorial office, Tel: +86-10-62920553, E-mail: jesc@263.net, jesc@rcees.ac.cn. Instruction to authors is available at http://www.jesc.ac.cn.

Journal of Environmental Sciences (Established in 1989)
Vol. 25 Supplement 2013

Supervised by	Chinese Academy of Sciences	**Published by**	Science Press, Beijing, China
Sponsored by	Research Center for Eco-Environmental		Elsevier Limited, The Netherlands
	Sciences, Chinese Academy of Sciences	**Distributed by**	
Edited by	Editorial Office of Journal of	Domestic	Science Press, 16 Donghuangchenggen
	Environmental Sciences		North Street, Beijing 100717, China
	P. O. Box 2871, Beijing 100085, China		Local Post Offices through China
	Tel: 86-10-62920553; http://www.jesc.ac.cn	Foreign	Elsevier Limited
	E-mail: jesc@263.net, jesc@rcees.ac.cn		http://www.elsevier.com/locate/jes
Editor-in-chief	Hongxiao Tang	**Printed by**	Beijing Beilin Printing House, 100083, China

CN 11-2629/X **Domestic postcode: 2-580** **Domestic price per issue RMB ¥ 110.00**

ISSN 1001-0742