JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Biodegradation of 3-Chloro-1,2-propanediol with *Saccharomyces cerevisiae*

RACHID BEL-RHLID, JEAN P. TALMON, LAURENT B. FAY, AND MARCEL A. JUILLERAT*

Nestlé Research Center, Nestec Ltd., Vers-Chez-les-Blanc, P.O. Box 44, 1000 Lausanne 26, Switzerland

A novel enzymatic dehalogenating activity of 3-chloro-1,2-propanediol (3-MCPD) with *Saccharomyces cerevisiae* (baker's yeast) is reported. All bioconversion assays were carried out under aerobic conditions, at 28 °C, and the kinetics were monitored. The biodegradation was performed at different pH values (6.2, 7.0, and 8.2), in the presence and absence of glucose, using racemic 3-MCPD at two different concentrations (7.3 μmol/L and 27 mmol/L). Optimal conversion (68%) of racemic (*R*,*S*)-3-MCPD at a concentration of 27 mmol/L was achieved after 48 h of reaction time, at pH 8.2, and in the presence of glucose. At a concentration of 7.3 μmol/L, 73% degradation was observed after 72 h, at pH 8.2 and in the absence of glucose. Under the same experimental conditions, the conversion of pure (*S*)-3-MCPD (85%) was higher than that of the (*R*)-enantiomer (60%).

KEYWORDS: 3-Chloro-1,2-propanediol; biodegradation; *Saccharomyces cerevisiae*

INTRODUCTION

3-Monochloro-1,2-propanediol (3-MCPD) is one of the numerous chlorinated food contaminants known collectively as chloropropanols. The identification, quantification, and mechanism of formation of 3-MCPD, particularly in processed foods, is well documented (*1*−*6*) and has recently been reviewed (*7*, *8*). Previous in vitro studies have shown that this halogenated compound has given positive results in in vitro mutagenicity studies and that it is an animal carcinogen, producing tumors at various sites in rats (*9*−*12*). As a consequence, several national and international regulatory agencies urged industry to reduce the level of 3-MCPD in all foods and ingredients to a minimum detectable level using the most sensitive methods available (*13*−*15*). For instance, the current approach in the U.K. is to ensure that the levels of 3-MCPD in foods and ingredients are <0.02 mg/kg based on 40% dry matter (*16*, *17*). However, a recent study concluded that a nongenotoxic mechanism for the carcinogenicity of 3-MCPD in rodents is plausible. These negative in vivo mutagenicity studies suggest that reactive metabolites, if formed, do not produce genotoxicity in the bone marrow and liver (*18*). Nevertheless, to achieve recommended levels of 3-MCPD, alternative technological processes were investigated. Thus, enzymatic hydrolysis of plant proteins instead of chemical hydrolysis and degradation of halogenated compounds with enzymes and microorganisms were established. In this last field, several microorganisms were identified as sources of enzymes which catalyze the transformation of halogenated compounds. The main investigations were focused on the enzymatic cleavage of the carbon−halogen bonds by whole-cell microorganisms (*19*−*21*) or by purified enzymes (*22*−*26*). These enzymatic activities were mainly identified in bacteria (e.g., *Flavobacteria*, *Corynebacteria*, and *Pseudomonas* sp.) (*27*−*30*) and fungi (e.g., *Caldariomyces fumago*) (*31*−*33*), and the five major enzymatic degradation pathways have been reviewed (*34*). Hardly any of these bacteria and fungi are food-grade, and therefore, their application in food products is not permitted. Thus, biodegradation of 3-MCPD with *Saccharomyces cerevisiae* was investigated, and the reaction parameters were studied. To our knowledge this is the first time that this biodegradation using whole-cell baker's yeast (*S. cerevisiae*) is reported.

MATERIALS AND METHODS

Materials. All chemicals and organic solvents were of analytical grade. 3-Monochloro-1,2-propanediol, (*S*)-(+)-3-monochloro-1,2-propanediol, and (*R*)-(−)-3-monochloro-1,2-propanediol were purchased from Aldrich (Steinheim, Germany). Sodium chloride, anhydrous sodium sulfate, anhydrous glucose, nitric acid (65%), and silver nitrate were obtained from Merck (Dietikon, Switzerland). d_5-3-Monochloro-1,2-propanediol was purchased from CDN Isotopes (Pointe-Claire, Canada) and heptafluorobutyrylimidazole (HFBI) from Pierce (Rockford, IL). Active dry baker's yeast was supplied by Hefe Schweiz (Stettfurt, Switzerland).

Preparation of Baker's Yeast. Dry baker's yeast (60 g) was added to 300 g of distilled water under magnetic stirring for 30 min at room temperature. The yeast cream was then centrifuged at 5000 rpm for 15 min (Beckman, model J2-21M) at 4 °C, and the supernatant was discarded. The yeast cells were resuspended in 225 mL of distilled water and the obtained yeast cream solution (pH 5.8) was then used for biodegradation trials.

* To whom correspondence should be addressed. E-mail: marcel-alexandre.juillerat@rdls.nestle.com. Phone: +41.21.785 8708. Fax: +41.21.785.8554.

10.1021/jf048980k CCC: $27.50
Published on Web 09/10/2004

Biodegradation Assays. The yeast cream solution (150 mL) was placed in a 250 mL flask equipped with an electrode and a magnetic stirrer (500 rpm). The flask was immersed in an oil bath at 28 °C. The suspension was adjusted to the desired pH, which was maintained throughout the reaction with a solution of 2 M sodium hydroxide using a Metrohm pH-stat device, model 691, equipped with a Metrohm Impulsomat 614 and Dosimat 665 (Metrohm, Herisau, Switzerland). The influence of the following parameters on the degradation of 3-MCPD was studied: pH (6.2, 7.0, and 8.2), carbon source (with or without glucose), substrate stereospecificity ((*S*)- and (*R*)-enantiomers), and substrate concentration (7.3 μmol/L and 27 mmol/L). All trials were performed under aerobic conditions and at 28 °C. For the reactions carried out in the presence of an energy source, 2 g of glucose was added to the reaction mixture at the initial time (0 h) and then 1 g after each 6 h of incubation.

A reaction control was performed for each biodegradation assay using the same experimental conditions, but without microbial cells to quantify the nonenzymatic transformation of 3-MCPD.

Extraction of 3-Monochloro-1,2-propanediol. 3-MCPD was extracted from the reaction medium using two techniques. The first one, which is commonly used, consisted of using solid-phase extraction based on a diatomaceous earth (Extrelut 3) column (*4*, *8*). For the second technique, a hydromatrix column was placed in an accelerated solvent extractor (ASE 200, Dionex, Olten, Switzerland), eluted with 80 mL of ethyl acetate, and the sample (2 mL of supernatant obtained as described above) was placed onto the column and extracted three times with 20 mL of ethyl acetate. The principle of this technique is similar to the use of diatomaceous earth (Extrelut), and the only difference is that the extraction time is dramatically reduced. To determine the recovery of 3-MCPD with the ASE technique, extraction of different standard solutions (different concentrations of 3-MCPD) was performed. Results showed that the concentration of 3-MCPD had no effect on the efficiency of the method, and that a recovery of 95% could be achieved with less than a 4% standard deviation. The experimental conditions used were the following: pressure, 11 bar; temperature, 25 °C; heat time, 1 min; purge time, 1 min; solvent, ethyl acetate; static time, 10 min; flush volume, 100%; number of static cycles, 1.

Analysis and Quantification of 3-MCPD. Derivatization of extracted 3-MCPD with heptafluorobutyrylimidazole (HFBI) was performed as described in the literature (*4*, *8*). The resulting 3-MCPD diesters were then analyzed by gas chromatography−mass spectrometry. This method has recently been validated (*35*), and the limit of the quantification has been established as being close to 10 ppb. The quantification of 3-MCPD was done by the stable isotope internal standard method using deuterium-labeled d_5-3-MCPD (*4*). Gas chromatography−mass spectrometry (GC−MS) analyses were performed on a MAT-8430 mass spectrometer (Finnigan, Bremen, Germany) combined with an HP gas chromatograph equipped with a split/splitless injector and fused silica capillary column, DB-5 (30 m × 0.32 mm i.d., film thickness 0.25 μm). The temperature program was 50 °C (1 min), 5 °C/min to 100 °C, 30 °C/min to 300 °C, 300 °C (2 min). The samples (2 μL) were injected in splitless mode, the injector temperature was 260 °C, and the transfer line temperature was set at 220 °C. Electron ionization (EI) was operated at 70 eV, and the ion source was set at 180 °C.

Chloride Titration. Chloride titration assays were carried out by direct potentiometric titration using a silver electrode and a standard silver nitrate solution as described in the literature (*36*). Each sample withdrawn from the reaction mixture (biodegradation of 3-MCPD by *S. cerevisiae*) was centrifuged (Sigma 3K12, Merck centrifuge) at 3000 rpm at 25 °C for 5 min. The aqueous phase (3 mL) was transferred to a 100 mL volumetric flask and made up to volume with distilled water. An aliquot (40 mL) of this solution was then transferred into a titration vessel, and 1 mL of concentrated nitric acid was added. The titration was performed with a solution of 0.01 N $AgNO_3$ using an automatic titrator (Combi-titrator Metrohm, including an Impulsomat 614, a piston buret Multi-Dosimat 645 with a stirrer, and a Dosigraph 625). The potentiometric end point was obtained by calculating the change in potential per unit volume of titrant. A plot of these data as a function of the average volume produced a curve with a maximum that corresponded to the inflection point. This method of end-point evaluation is based on the assumption that the titration curve is symmetric about the equivalence point and that the inflection in the curve corresponds to this point.

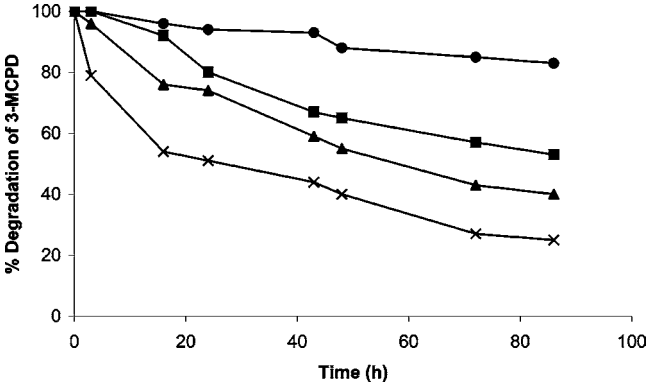

Figure 1. Effect of pH and concentration on the biodegradation of 3-MCPD with *S. cerevisiae*. Substrate concentration 27 mmol/L: (●) pH 6.2, (■) pH 7.0, (▲) pH 8.2. Substrate concentration 7.3 μmol/L: (×) pH 8.2.

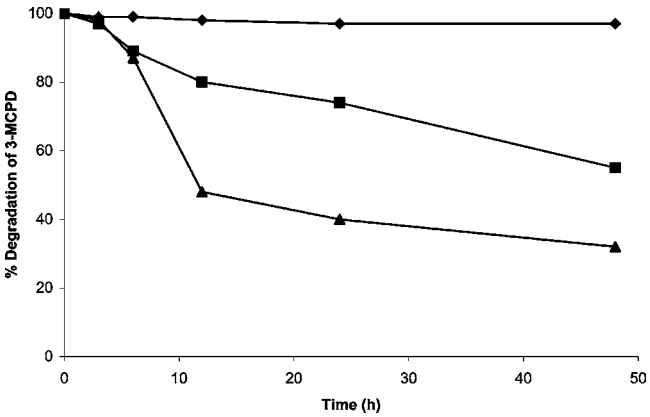

Figure 2. Influence of the energy source on the biodegradation rate and yield of 3-MCPD with *S. cerevisiae*. Reactions performed under aerobic conditions, at pH 8.2 and 28 °C. Key: (■) absence of glucose, (▲) presence of glucose, (◆) control.

RESULTS

Effect of pH and Substrate Concentration. Reactions were performed at three pH values (6.2, 7.0, and 8.2) using racemic 3-MCPD at a concentration of 27 mmol/L. All incubations were carried out under aerobic conditions at 28 °C without addition of glucose. Control vials containing reaction medium and substrate, but not the microorganism, were run alongside the trials. As shown in **Figure 1**, 3-MCPD was transformed at all pH values studied and the maximum biodegradation was reached at pH 8.2. At this pH, 45% of 3-MCPD was converted after 48 h of reaction time, while only 35% and 12% transformations were observed at pH 7.0 and 6.2, respectively. The control trials showed less than 3% degradation at the three pH values investigated. At a low concentration (7.3 μmol/L) and pH 8.2, 60% of 3-MCPD was transformed after 48 h of reaction time (**Figure 1**).

Biodegradation in the Presence of Glucose. The addition of glucose as a carbon source enhanced the 3-MCPD degradation rate. Thus, 68% of the substrate was transformed after 48 h of incubation in the presence of glucose, while only 45% could be achieved in the absence of glucose at the same reaction time (**Figure 2**). These reactions were performed under aerobic conditions at pH 8.2 and 28 °C using racemic 3-MCPD at a concentration of 27 mmol/L. The control trials done in the

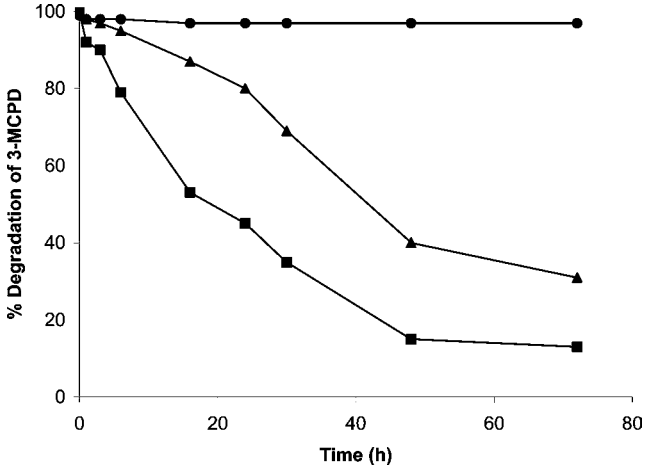

Figure 3. Kinetic study of the transformation of both 3-MCPD enantiomers with *S. cerevisiae* at pH 8.2 and 28 °C, under aerobic conditions, and in the absence of glucose. Key: (■) (*S*)-3-MCPD, (▲) (*R*)-3-MCPD, (●) control.

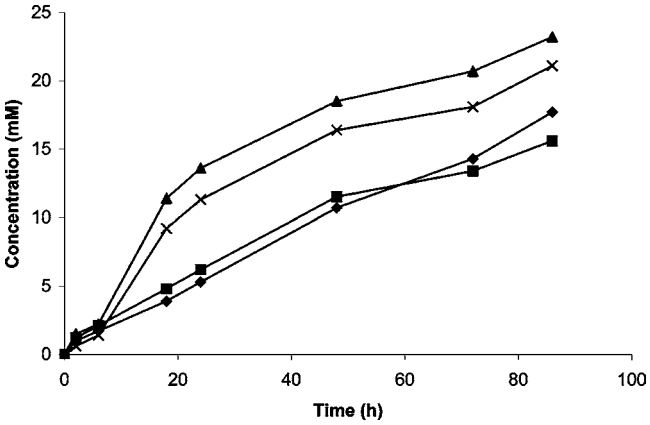

Figure 4. Comparison of the concentration of chloride ions liberated and 3-MCPD transformed with *S. cerevisiae* under aerobic conditions and in the absence of glucose. Chloride ions liberated at (◆) pH 7.0 and (▲) pH 8.2. 3-MCPD degraded at (■) pH 7.0 and (×) pH 8.2.

absence of microorganism again showed less than 3% degradation of 3-MCPD.

Enantioselective Biotransformation. Pure (*R*)- and (*S*)-3-MCPD enantiomers were degraded under aerobic conditions at pH 8.2 and 28 °C to evaluate the stereoselectivity of the enzymatic system involved in this biotransformation. Results showed that the (*S*)-enantiomer was degraded at a higher rate and percentage in comparison to the (*R*)-enantiomer (**Figure 3**). A maximum degradation of 85% was achieved with (*S*)-3-MCPD while only 60% degradation was observed for the (*R*)-enantiomer after 48 h of reaction time.

Chloride Titration. To confirm that the degradation of 3-MCPD with *S. cerevisiae* is due to an enzymatic reaction, the amount of chloride released in the reaction medium was compared to the amount of 3-MCPD transformed. Potentiometric titration of chloride was performed on samples withdrawn from biodegradation trials at pH 7.0 and 8.2. The kinetics of the consumption of 3-MCPD and release of chloride in the reaction medium are reported in **Figure 4**. Each measurement of chloride content represents the average of three replicates, with a mean standard deviation of less than 2%. These results and the control trial without yeast cells (less than 3% degradation) suggest that the degradation of 3-MCPD is due to enzymatic reactions and not to another physicochemical phenomenon such as adsorption for instance.

DISCUSSION

S. cerevisiae is a food-grade microorganism that has proven to be an extremely valuable source of enzymes for biocatalytic applications. It is a multienzyme system which eventually gives rise to multistep sequential reactions. However, the presence of a nonconventional substrate in the complex enzymatic system can cause a number of different responses at the same time, which could be responsible for unexpected substrate transformations (*37*). Because of these specificities, we investigated the degradation of 3-MCPD with baker's yeast. Kinetic studies were performed at three pH values (6.2, 7.0, and 8.2) which were reported as optimal pH values for some dehalogenating enzymes. In fact, the maximal dechlorinating activity of 3-MCPD with an enzyme from *Alcaligenes* sp. was obtained at pH 7.3, and this enzyme was found to be stable in the pH range 6.0−8.0 (*20*). It has also been reported that the optimal activity of haloalkane dehalogenase from *Xanthobacter autotrophicus* GJ10 was observed at pH 8.2 (*38*). In our investigation, maximal dechlorinating activity of baker's yeast toward 3-MCPD was reached at pH 8.2 (45%) after 48 h of reaction time. The assays were repeated three times, and the results showed a good reproducibility (less than 2% standard deviation).

The degradation of 3-MCPD with *S. cerevisiae* took place over a long time period (>48 h), which indicates that the substrate may simply be assimilated by the cells as a carbon source instead of being dehalogenated. To avoid this possibility, small amounts of glucose were added dropwise to the reaction mixture as an energy source. The addition of glucose resulted in an enhancement of the reaction rate and yield. Thus, 60% of 3-MCPD (27 mmol/L) could be transformed after 24 h of reaction time, whereas only 25% was converted at the same time in the absence of glucose.

Since the conversion of racemic 3-MCPD was not complete even at low concentration (7.3 μmol/L) and after 3 days of incubation, the hypothesis of an enantioselective biodegradation was envisaged. Moreover, a previous study showed that a *Pseudomonas* strain degraded stereoselectively the enantiomer (*S*)-3-MCPD (*39*). It has also been reported that the dehalogenating activity of an *Alcaligenes* sp. toward (*R*)-3-MCPD was especially high compared to that toward the (*S*)-enantiomer (*20*). In our investigation, *S. cerevisiae* transformed the enantiomer (*S*)-3-MCPD at a higher rate in comparison to the (*R*)-enantiomer. In fact, 85% of (*S*)-3-MCPD was degraded after 48 h, whereas only 60% conversion was achieved with (*R*)-3-MCPD after the same reaction time. This dehalogenation process with whole-cell baker's yeast may involve one enzyme degrading, in a nonstereoselective way, both enantiomers but having a kinetic preference toward (*S*)-3-MCPD. Alternatively, the process may also involve two different enzymes acting specifically on each enantiomer with different conversion rates.

It is obvious that, to better understand this new dehalogenating activity of *S. cerevisiae* and to establish the biodegradation pathways, the involved enzymes as the reaction intermediates and products have to be further isolated and characterized. Knowledge of the basic metabolic pathways in yeast fermentation is well established, but for the biotransformation of nonnatural or xenobiotic substrates only very little and nonsystematic information is available.

The new dehalogenating activity of *S. cerevisiae* reported here opens new perspectives for the degradation of halogenated

contaminants. The food-grade characteristics, ease of use, and low cost of baker's yeast, compared to other microorganisms, present real advantages for prospective industrial applications.

ACKNOWLEDGMENT

We are grateful to S. Metairon for her expert technical assistance and to E. Prior for her critical review of the manuscript.

LITERATURE CITED

(1) Breitling-Utzmann, C. M.; Köbler, H.; Herbolzheimer, D.; Maier, A. 3-MCPD-occurrence in bread crust and various food groups as well as formation in toast. *Dtsch. Lebensm.-Rundsch*. **2003**, *99*, 280−285.

(2) Collier, P. D.; Cromie, D. D. O.; Davies A. P. Mechanism of formation of chloropropanols present in protein hydrolysates. *J. Am. Oil Chem. Soc*. **1991**, *68*, 785−790.

(3) Crews, C.; Brereton, P. P.; Davies, A. Effect of domestic cooking on the levels of 3-monochloropropane-1,2-diol in food. *Food Addit. Contam.* **2001**, *18*, 271−280.

(4) Hamlet, Colin. G. Analytical methods for the determination of 3-chloro-1,2-propandiol and 2-chloro-1,3-propandiol in hydrolysed vegetable protein, seasonings and food products using gas chromatography/ion trap tandem mass spectrometry. *Food Addit. Contam*. **1998**, *15*, 451−465.

(5) Macarthur, R.; Crews, C.; Davies, A.; Brereton, P.; Hough, P.; Harvey, D. 3-MCPD in soy sauces and similar products available from retail outlets in the UK. *Food Addit. Contam*. **2000**, *17*, 903−906.

(6) Meirerhans, R.; Bruehlmann, S.; Meili, J.; Taeschler, C. Sensitive method for the determination of 3-chloropropane-1,2-diol and 2-chloropropane-1,3-diol by capillary gas chromatography with mass spectrometric detection. *J. Chromatogr. A* **1998**, *802*, 325−333.

(7) Crews, C.; Hough, P.; Brereton, P.; Harvey, D.; Macarthur, R.; Matthews, W. Survey of 3-monochloropropane-1,2-diol (3-MCPD) in selected food groups, 1999−2000. 2002. *Food Addit. Contam*. **2002**, *19*, 22−27.

(8) Hamlet, Colin, G.; Sadd, P. A.; Crews, C.; Velišek, J.; Bexter, E. Occurrence of 3-chloro-1,2-propane-diol (3-MCPD) and related compounds in food: a review. *Food Addit. Contam.* **2002**, *19*, 619−631.

(9) Committee on carcinogenicity of chemicals in food, consumer products and the environment. *Statement on carcinogenicity of 3-mono- chloropropane-1,2-diol (3-MCPD)*; COM/00/S4; 2000 [online] http://www.doh.gov.uk/mcpd2.htm.

(10) Committee on carcinogenicity of chemicals in food, consumer products and the environment. *Statement on carcinogenicity of 3-monochloropropane-1,2-diol (3-MCPD)*; COC/00/S5; 2000 [online] http://www.doh.gov.uk/mcpd1.htm.

(11) Lynch, B. S.; Bryant, D. W.; Hook, G. J.; Nestmann, E. R.; Munro, I. C. Carcinogenicity of monochloro-1,2-propanediol (alpha-chlorohydrin, 3-MCPD). *Int. J. Toxicol.* **1998**, *17*, 47−76.

(12) Zeiger, E.; Anderson, B.; Haworth, S.; Lawlor, T.; Mortelmans, K. *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environ. Mol. Mutagen*. **1998**, *11*, 1−158.

(13) European Commission. *Directive on the quality of water intended for human consumption*; Council Directive 98/83/EC; European Commission: Brussels, Belgium, Nov 3, 1998.

(14) European Commission. *Opinion of the scientific committee on food on 3-monochloropropane-1,2-diol (3-MCPD) updating the SCF opinion of 1994*; SCF/CS/CNTM/OTH/17 Final; European Commission: Brussels, Belgium, May 2001.

(15) Food Advisory Committee. *(iv) 3-MCPD FAC Press Release 8/100*; 2000 [online] http://www.foodstandards.gov.uk/committees/fac/press_releases.htm.

(16) Commission Regulation (EC) No. 466/2001. Setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur. Communities* **2001**, L77.1.

(17) Joint FAO/WHO Expert Committee on Food Additives. *Summary of the fifty-seventh meeting of the Joint FAO/WHO Expert Committee on food additives (JECFA)*, Rome, Italy, June 5−14, 2001; pp 20−24.

(18) Robjohns, S.; Marshall, R.; Fellows, M.; Kowalczyk, G. *In vivo* genotoxicity studies with 3-monochloropropan-1,2-diol. *Mutagenesis* **2003**, *18*, 401−404.

(19) Effendi, A. J.; Greenaway, S. D.; Dancer, B. N. Isolation and characterisation of 2,3-dichloro-1-propanol-degrading rhizobia. *Appl. Environ. Microbiol*. **2000**, *66*, 2882−2887.

(20) Suzuki, T.; Kasai, N.; Yamamoto, R.; Minamiura, N. A novel enzymatic dehalogenation of (*R*)-3-chloro-1,2-propanediol in *Alcaligenes* sp. DS-S-7G. *Appl. Microbiol. Biotechnol*. **1994**, *42*, 270−279.

(21) Van den Vijngaard, A. J.; Janssen, D. B.; Witholt, B. Degradation of epichlorohydrin and halohydrins by bacterial cultures isolated from fresh-water sediments. *J. Gen. Microbiol*. **1989**, *135*, 2199−2208.

(22) Leisinger, T.; Bader, R. Microbial dehalogenation of synthetic organohalogen compounds: hydrolytic dehalogenases. *Chimia* **1993**, *47*, 116−121.

(23) Petri, A.; Gambicorti, T.; Salvadori, P. Covalent immobilization of chloroperoxidase on silica gel and properties of the immobilized biocatalyst. *J. Mol. Catal. B: Enzym*. **2004**, *27*, 103−106.

(24) Schanstra, J. P.; Kingma, J.; Janssen, D. B. Specificity and kinetics of haloalkane dehalogenase. *J. Biol. Chem*. **1996**, *271*, 14747−14753.

(25) Van den Wijngaard, A. J.; Reuvekamp, P. T. W.; Janssen, D. B. Purification and characterization of haloalcohol dehalogenase from *Artherobacter* sp. AD2. *J. Bacteriol*. **1991**, *173*, 124−129.

(26) Verschueren, Koen. H. G.; Seljee, F.; Rozeboom, H. J.; Kalk, K. H.; Dijkstra, B. W. Crystallographic analysis of the catalytic mechanism of haloalkane dehalogenase. *Nature* **1993**, *363* (6431), 693−698.

(27) Geigert, J.; Neidleman, S. L.; Dalietos, D. J.; Dewitt, S. K. Novel haloperoxidase reaction: synthesis of dihalogenated products. *Appl. Environ. Microbiol*. **1983**, *45*, 1575−1581.

(28) Nakamura. T.; Nagasawa, T.; Yu, F.; Watanabe, I.; Yamada, H. Resolution and some properties of enzyme involved in enantioselective transformation of 1,3-dichloro-2-propanol to (*R*)-3-chloro-1,2-propanediol by *Corynebacterium* sp. strain N-1074. *J. Bacteriol*. **1992**, *174*, 7613−7619.

(29) Slater, J. H.; Bull, A. T.; Hardman, D. J. Microbial dehalogenation of halogenated alcanoic acids, alcohols and alkanes. *Adv. Microb. Physiol*. **1997**, *38*, 133−176.

(30) Suzuki, T.; Kasai, N.; Yamamoto, R.; Minamiura, N. Isolation of a bacterium assimilating (*R*)-3-chloro-1,2-propanediol and production of (*S*)- 3-chloro-1,2-propanediol using microbial resolution. *J. Ferment. Bioeng*. **1992**, *73*, 443−448.

(31) Camilo, E.; La Rotta, H.; Elba, P. S. Bon. 4-Chlorophenol degradation by chloroperoxidase from *Caldariomyces fumago*. *Appl. Biochem. Biotechnol*. **2002**, *98*, 191−203.

(32) Hollenberg, P. F.; Hager, L. P. Purification of chloroperoxidase from *Caldariomyces fumago*. *Methods Enzymol*. **1978**, *52*, 521−529.

(33) Pickard, M. A.; Hashimoto, A. Stability and carbohydrate composition of chloroperoxidase from *Caldariomyces fumago* grown in a fructose-salts medium. *Can. J. Microbiol*. **1988**. *34*, 998−1002.

(34) Faber, K. Halogenation and dehalogenation reactions. In *Biotransformations in Organic Chemistry*, 3rd ed. (a textbook); Faber, K., Ed.; Springer-Verlag: Germany, 1997; pp 288−299.

(35) Brereton, P.; Kelly, J.; Crews, C.; Honour, S.; Wood, R.; Davies, A. Determination of 3-monochloropropane-1,2-diol in food and food ingredients using mass spectrometric detection: collaborative study. *J. AOAC Int*. **2001**, *84*, 1−11.

(36) Preen, M. A.; Woodward, J. D. Potentiometric titration of chloride in beer and wort using an ion-selective electrode. *J. Inst. Brew*. **1975**, *81*, 307−308.
(37) Servi, S. In *Biotechnology*, 2nd ed.; Rehm, H. J., Reed, G., Eds.; *Vol. 8a: Biotransformations I*; Kelly, D. R., Ed.; Wiley-VCH: New York, 1998; pp 363−389.
(38) Verschueren, Koen. H. G.; Franken, S. M.; Rozeboom, H. J.; Kalk, K. H.; Dick, B.; Dijkstra, B. W. Refined X-ray structures of haloalkane dehalogenase at pH 6.2 and pH 8.2 and implications for the reaction mechanism. *J. Mol. Biol*. **1993**, *232*, 856−872.
(39) Suzuki, T.; Kasai, N.; Yamamoto, R.; Minamiura, N. Production of highly optically active (*R*)-3-chloro-1,2-propanediol using a bacterium assimilating the (*S*)-isomer. *Appl. Microbiol. Biotechnol*. **1993**, *40*, 273−278.

Received for review June 23, 2004. Revised manuscript received August 4, 2004. Accepted August 5, 2004.

JF048980K