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Biodegradation of 1,2,3-Trichloropropane to Valuable (*S*)-2,3-DCP Using a One-Pot Reaction System

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Abstract: 1,2,3-trichloropropane (TCP) being one of the important environmental pollutants, has drawn significant concern due to its highly toxic and carcinogenic effects. In this study, we built a one-pot reaction system in which immobilized haloalkane dehalogenase (DhaA31) and halohydrin dehalognase (HheC) were used to catalyze the recalcitrant TCP to produce 2,3-dichloro-1-propanol (2,3-DCP) by removing epichlorohydrin (ECH). Since HheC displays a high *R* enantiopreference toward 2,3-DCP, the production of enantiopure (*S*)-2,3-DCP was expected. However, the enantioselective resolution of (*R*,*S*)-2,3-DCP by HheC was greatly inhibited by the circular reaction occurring between the product ECH and 1,3-dichloro-2-propanol (1.3-DCP). To resolve this problem, HZD-9 resin-based in situ product removal was implemented. Under the optimized conditions, TCP was completely consumed, resulting in optically pure (*S*)-2,3-DCP with enantiomer excess (*e.e*) > 99% and 40% yield (out of the 44% theoretical maximum). The scale-up resin-integrated reaction system was successfully carried out in 0.5 L batch reactor. Moreover, the system could be reused for 6 rounds with 64% of original activity retained, showing that it could be applied in the treatment of large volumes of liquid waste and producing enantiopure (*S*)-2,3-DCP.

Keywords: immobilized dehalogenases; 1,2,3-trichloropropane; enantiopure compound; one-pot reaction; in-situ product removal

1. Introduction

1,2,3-trichloropropane (TCP) is a xenobiotic halocarbon, which has many industrial and agricultural applications, such as a solvent for degreasing, a substrate for the synthesis of polysulfone liquid polymers, hexafluoropropylene, and polysulfides, and a precursor in soil fumigants [1,2]. Because of its low soil organic carbon-water partition coefficient, TCP is unlikely to adsorb in soil and readily moves to ground water reservoirs [1], which makes TCP a stable water contaminant.

TCP has garnered significant concern due to its highly toxic and carcinogenic effects [2–4]. Therefore, cleanup of TCP contaminated sites using biodegradation systems is of paramount importance. Bosma and co-workers introduced a synthetic metabolic pathway by applying heterologously expressed haloalkane dehalogenase (DhaA) from Rhodococcus sp. in the natural host *Agrobacterium radiobacter* AD1, which can metabolize haloalcohols [5]. However, the efficiency of the system was low, and it was

insufficient for use as a biocatalyst. For this, a mutant DhaA31 that showed a 32-fold improvement in activity was evolved by redesigning the dehalogenase access tunnels [6]. An in vitro five-stage metabolic pathway was built to mineralize TCP to the harmless product glycerol [2,7]. In this degradation pathway, the reaction intermediate (S)-2,3-DCP was accumulated in the system due to the low activity of HheC on (S)-2,3-DCP, and a prolonged reaction time was needed to completely degrade it. However, (S)-2,3-DCP is an important chiral compound which has many applications in pharmaceuticals and fine chemical processing [8,9]. Alternatively, van Leeuwen and co-workers tried to degrade TCP to a highly enantioenriched (R)- or (S)-2,3-DCP by using enantio-complementary haloalkane dehalogenase variants obtained by directed evolution [10]. The resulting mutants could convert TCP to (R) and (S)-2,3-DCP with enantiomer excess (*e.e.*) of 90% and 97%, respectively. Even though the *e.e.* value of both mutants is higher in comparison to both the wild type and DhaA31 enzymes, their activities were much lower than the so far most active haloalkane dehalogenase mutant DhaA31. This greatly hampered their applications as biocatalysts to produce enantiopure (R)-2,3-DCP and (S)-2,3-DCP.

Halohydrin dehalogenase (HheC) has been used for the kinetic resolution of optically pure haloalcohols [8,11,12]. By modifying and optimizing the biodegradation pathway used by Dvorak and co-workers [2], it is possible to produce pure (S)-2,3-DCP through this system. However, the liberated Cl- opens the epoxide and as a consequence 1,3-dichloro-2-propanol (1,3-DCP) was formed. The referenced dehalogenation reaction of HheC toward 1,3-DCP greatly inhibits the enzymatic activity on 2,3-DCP [9]. Therefore, it needs a long time to completely consume the R enantiomer of 2,3-DCP, which shows negative effects on the enantiopurity of (S)-2,3-DCP. Thus, in order to obtain optically pure (S)-2,3-DCP, the above circular reaction needs to be blocked. In the kinetic resolution of 2,3-DCP using HheC in a whole cell format, an aqueous-organic biphasic system was used to solve such a problem [8]. On the other hand, resin-based in situ product removal could be another option to resolve such an inhibition. The resin HZD-9 has been successfully applied in the synthesis of ECH from 1,3-DCP catalyzed by HheC [13,14].

On the other hand, applying free enzymes as a biocatalyst is not ideal due to the difficulty of reuse, low stability in harsh conditions like high mechanical forces, high temperature, and organic solvents. HheC has been successfully immobilized using the cross-linking enzyme aggregates technique (CLEAs). The resulting immobilized enzyme HheC-CLEAs showed higher activity and stability [15]. The application of HheC-CLEAs along with immobilized DhA31 in biotransformation of TCP to valuable compounds could be a promising way. Here, we introduced resin-based in situ product removal batch reaction process to produce enantiopure (S)-2,3-DCP from TCP (Figure 1) using an immobilized DhaA31 (DhaA31-CLEAs) and HheC (HheC-CLEAs).

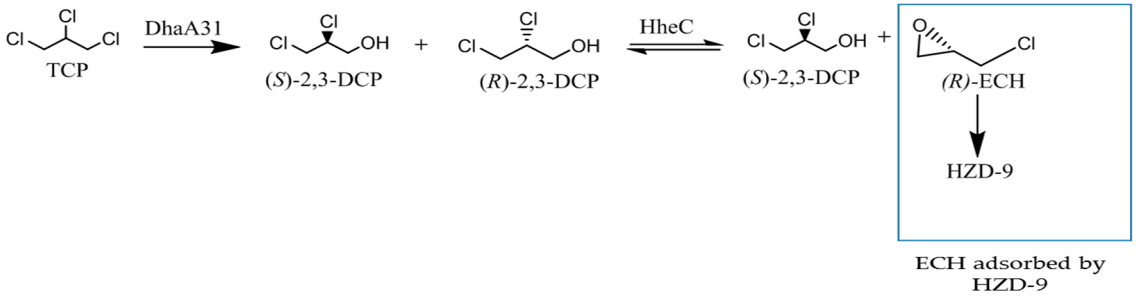

Figure 1. Scheme of resin-integrated one-pot reaction system for the synthesis of enantiopure (*S*)-2,3-DCP from 1,2,3-trichloropropane (TCP).

2. Results and Discussion

2.1. Identification of Bottlenecks in Degradation of TCP to Produce (S)-2,3-DCP

In order to produce optically pure (*S*)-2,3-DCP, the reaction system was initially setup by incubating 5 mM TCP in 10 mL total reaction mixture, using 1 mg each of DhaA31-CLEAs and HheC-CLEAs (1:1 mass ratio). The TCP conversion was performed in 50 mM phosphate buffer (pH 8) at 37 °C. As shown in Figure 2, TCP was fully degraded after 6.5 h, while both (*R*)-2,3-DCP and (*S*)-2,3-DCP were observed in the system. As HheC has a very high enantioselectivity (E > 100) for the (*R*)-2,3-DCP [9], it is expected to produce enantiopure (*S*)-2,3-DCP in the system. In contrast to that, a product with low *e.e.* of 49% was obtained with a yield of 44% (*S*)-2,3-DCP. Such a low *e.e.* value of (*S*)-2,3-DCP could be due to the reverse reaction of ECH as shown in Supplementary Figure S1 [9]. This racemization has vital impact on the activity of HheC toward 2,3-DCP and subsequently to the enantiopurity of (*S*)-2,3-DCP. To overcome this limitation, ECH removal was proposed as a possible solution. For this, an aqueous-organic biphasic system and resin-mediated product removal were successfully applied in some cases [8,13,14].

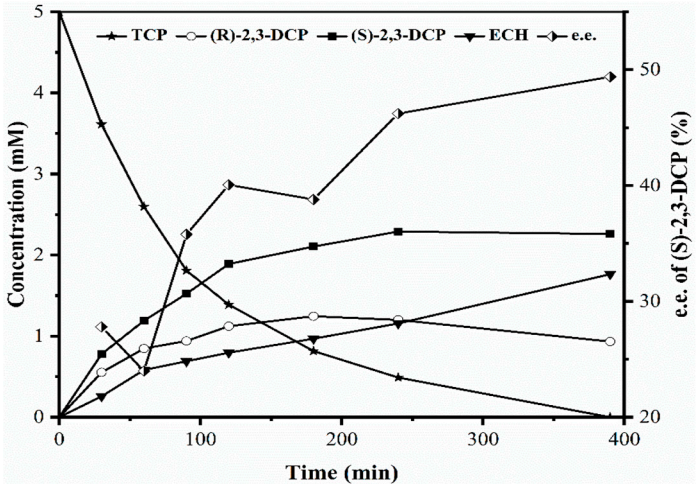

Figure 2. The time course of a one-pot degradation of TCP using immobilized DhaA31-CLEAs and HheC-CLEAs. Reaction conditions: the reaction mixture contained 10 mL phosphate buffer (50 mM, pH 8) containing 5 mM TCP, 1 mg DhaA31-CLEAs, and 1 mg HheC-CLEAs, incubated for 6.5 h in a screw-capped 20 mL bottle at 37 °C.

In this study, we evaluated the suitability of the above-mentioned approaches to solve the racemization problem. As shown in Figure S2, the reaction performed in n-heptane-aqueous bi-phase system (20%, v/v) was relatively less efficient than the aqueous phase reaction. Under the same experimental conditions with the aqueous phase system, only 60% of TCP degradation and 75% *e.e.* of (*S*)-2,3-DCP were achieved even after more than 23 h incubation. Such lesser efficiency of TCP degradation could be due to the high organic carbon-water partition coefficient of TCP (log K_{oc} = 2.27). Moreover, with the bi-phase system the reverse reaction of ring-opening of (*R*)-ECH to form 1,3-DCP was not significantly inhibited, resulting in such a low *e.e.* value of (*S*)-2,3-DCP. In our case, the resin-mediated product removal was proved more promising than the bi-phasic system. Since resin-based ECH removal for cell-free system has been successfully applied to the biotransformation of 1,3-DCP to ECH [13,14], resin (HZD-9) mediated in situ product removal was considered for the subsequent experiments.

2.2. Optimization of Resin-Integrated One-Pot Degradation System

2.2.1. Effect of HZD-9 Concentration on Biotransformation

In resin-mediated biotransformation, the optimum amount of resin is of paramount importance. We tested the effect of the amount of resin HZD-9 (%) (w/v) on enzyme activity. HZD-9 was applied previously to selectively remove ECH [13,14]. As shown in Figure 3, no significant effect of HZD-9 (up to 5%) on the enzymatic activity was observed. TCP was completely degraded in 6 h and the *e.e.* of (*S*)-2,3-DCP was higher than 99%. While at 10% HZD-9, a significant inhibition on the activity of DhaA31-CLEAs was observed. Only 75% TCP was degraded after 6 h. Therefore, 5% HZD-9 was selected as an optimum concentration and used in the subsequent experiments.

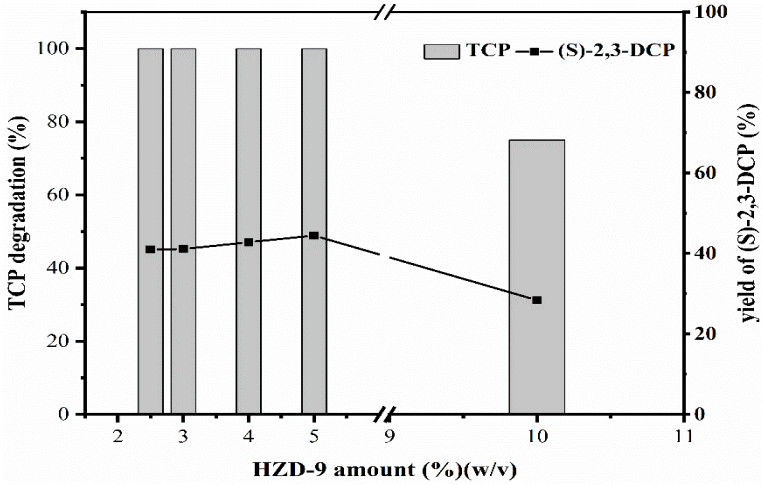

Figure 3. The effect of HZD-9 resin on enhancing the *e.e.* and yield of (*S*)-2,3-DCP. Reaction conditions: a reaction mixture of 10 mL borate buffer (0.2 M, pH 8.5) containing 5 mM TCP, 1 mg DhaA31-CLEAs, 1 mg HheC-CLEAs, 5 samples with differing added mass of HZD-9 resin (w/v %) (2.5%, 3%, 4%, 5%, and 10 %), incubated at 37 °C and 180 rpm for 6 h in a screw-capped 20 mL bottle.

2.2.2. Effect of Temperature on Biotransformation

The effect of temperature on the biotransformation of TCP was tested at a temperature range from 25 °C to 50 °C. Since temperature not only influenced the enzyme activities, but also affected enantioselectivity, stability, and the equilibrium of a reaction in some cases [16]. As shown in Figure 4, the degradation of TCP increased with an increase in temperature of the reaction up to 37 °C. Further increase in temperature did not exhibit any significant increased effect on both the degradation of TCP and the formation of (*S*)-2,3-DCP. Therefore, the reaction temperature of 37 °C was regarded as the optimum one.

2.2.3. Effect of pH on Biotransformation

Enzyme based dehalogenation reaction is highly pH dependent. It plays a vital role by maintaining the ionic environment of enzymes [17]. As shown in Figure 5, the TCP degradation efficiency increased with an increase in pH within a range of 7.0 to 9.0 from 53% to 82% within 3 h. However, the production of (*S*)-2,3-DCP also increased with an increase in pH (7.0–9.0), the yield of (*S*)-2,3-DCP was decreased with further increase in pH above 8.5. Similar effect was observed by Zou et al. where an increase in pH of the reaction system led to an increase in enzyme activity and decrease in yield [8]. Hence, pH 8.5 was selected for subsequent study.

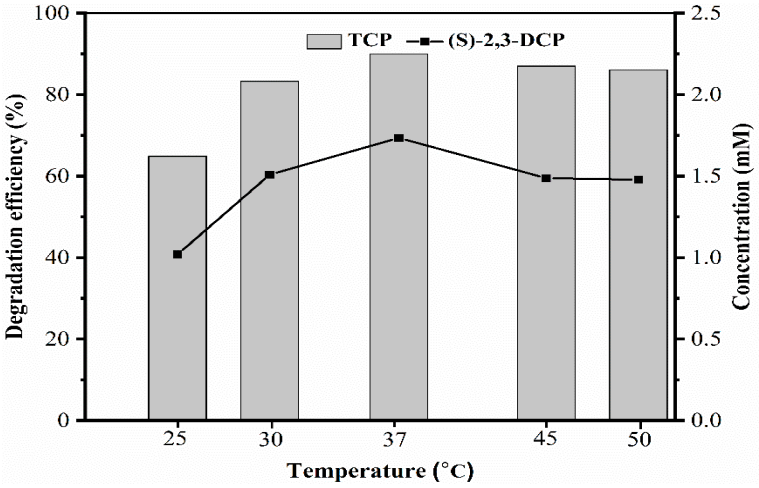

Figure 4. Influence of temperature on the biodegradation of TCP, the *e.e.* and yield of (*S*)-2,3-DCP in a resin-based integrated system. Reaction conditions: reaction mixture of 10 mL borate buffer (0.2 M, pH 8.5) containing 5 mM TCP; 1 mg DhaA31-CLEAs, 1 mg HheC-CLEAs, and 0.5 g HZD-9 resin, incubated for 3 h in a screw-capped 20 mL bottle at different temperature and 180 rpm.

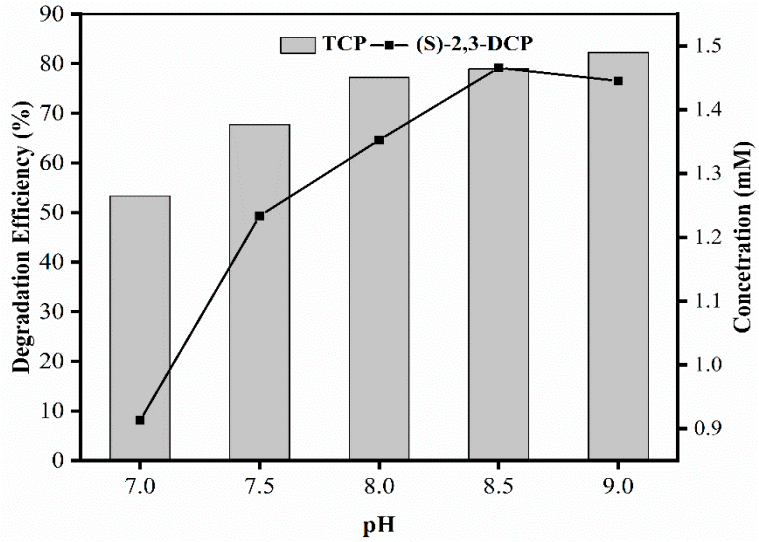

Figure 5. Influence of pH on the biodegradation of TCP, *e.e.* and yield of (*S*)-2,3-DCP in a resin-based integrated system. Reaction conditions: a reaction mixture of 10 mL buffer (phosphate buffer (0.2 M, pH [7.0–8.0]), borate buffer (0.2 M, pH [8.0–9.0])), containing 5 mM TCP; 1 mg DhaA31-CLEAs, 1 mg HheC-CLEAs, and 0.5 g HZD-9 resin, incubated for 3 h in a screw-capped 20 mL bottle at 37 °C and 180 rpm.

2.2.4. Effect of Enzyme Concentration on Biotransformation

In order to explore the effect of enzyme concentration on the degradation rate of TCP, *e.e.* and the yield of (*S*)-2,3-DCP, the amount of DhaA31-CLEAs and HheC-CLEAs used in the system were tested in a single factor factorial design by keeping one as constant (with excess amount) and changing the other, while the rest of the parameters were kept the same as the above experiments. As shown in Figure 6a,b within a reaction time of 6 h, an increase in the concentration of DhaA31-CLEAs showed both improvement in the degradation efficiency of TCP and the yield of (*S*)-2,3-DCP, which reached at the maximum values with 2 mg of enzyme. Thus, 2 mg DhaA31-CLEAs was considered as the optimum enzyme concentration. As shown in Figure 6b an increment in HheC-CLEAs up to 0.75 mg led to a slight decrease in the yield but a significant increase in the *e.e.* value of (*S*)-2,3-DCP. The highest value of *e.e.* > 99% was achieved with 0.75 mg of HheC-CLEAs. Further increase in HheC-CLEAs led

to a slight decrease in yield. Thus, the optimum concentration of HheC-CLEAs was maintained at 0.75 mg.

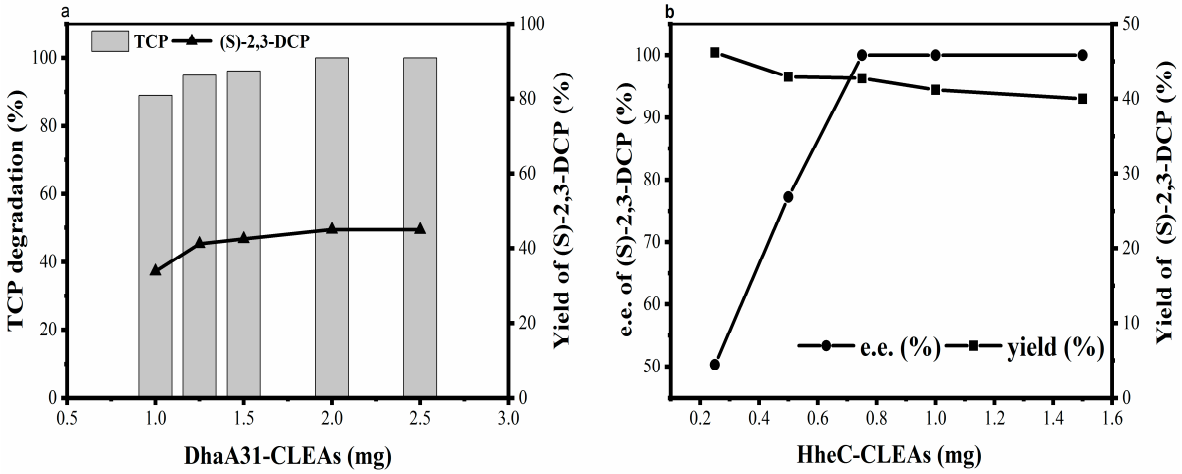

Figure 6. Influence of enzyme concentration on the biodegradation of TCP, *e.e.* and yield of (*S*)-2,3,-DCP in a resin-based integrated system. Reaction conditions: (**a**) reaction mixture of 10 mL borate buffer (0.2 M, pH 8.5) containing 5 mM TCP, 1 mg HheC-CLEAs, 0.5 g HZD-9 resin, and different weight of DhaA31-CLEAs, incubated for 3 h in a screw-capped 20 mL bottle at 37 °C and 180 rpm. (**b**) reaction mixture of 10 mL borate buffer (0.2 M, pH 8.5) containing 5 mM TCP; 2 mg DhaA31-CLEAs, 0.5 g HZD-9 resin, and different weight of HheC-CLEAs, incubated for 3 h in a screw-capped 20 mL bottle at 37 °C and 180 rpm.

2.2.5. Resin-Integrated One-Pot Biotransformation Performed at The Optimized Conditions

After a systematic optimization of reaction conditions, the optimized conditions were applied to the batch biodegradation of 5 mM TCP in 10 mL reaction volume and kinetic resolution of (*R*,*S*)-2,3-DCP in the presence of HZD-9 resin. As shown in Figure 7, TCP was completely degraded within 5 h. The (*S*)-2,3-DCP was obtained with the *e.e.* > 99% and 40% yield. The yield of (*S*)-2,3-DCP is mainly dependent on the degradation of TCP, while the *e.e.* value of (*S*)-2,3-DCP depends on the circular reaction between (*R*)-2,3-DCP and ECH. By the aid of HZD-9 in situ product removal, the *e.e.* of (*S*)-2,3-DCP was improved to more than 99%. The removal of ECH from the reaction system greatly improved HheC-CLEAs activity on (*R*)-2,3-DCP and shortened the reaction time to 5 h to achieve the highest purity of (*S*)-2,3-DCP. The obtained 40% yield correlated well with the reported theoretical yield of 44%; since DhaA31 converts TCP to R and (*S*)-2,3-DCP with an *e.e.* equal to 13% of (*R*)-2,3-DCP [18]. Although it has been reported that the adsorbed ECH to the HZD-9 could be fully recovered [13], it did not work in our case.

2.2.6. Scaled Up Batch Biodegradation of TCP to (S)-2,3-DCP

The scaled-up performance of this experiment was evaluated by using a 100 mL reaction system in a 500 mL shaking flask using a rotary shaker at 37 °C and 180 rpm under the optimized conditions. As shown in Figure 8, all the progress curve showed a similar trend as that performed in a 10 mL reaction system, resulting in optically pure (*S*)-2,3-DCP (*e.e.* > 99%). The yield of (*S*)-2,3-DCP was 39.5%, which is comparable with the previous experiments. The results indicated that the reaction volume did not show a clear influence on the reaction properties.

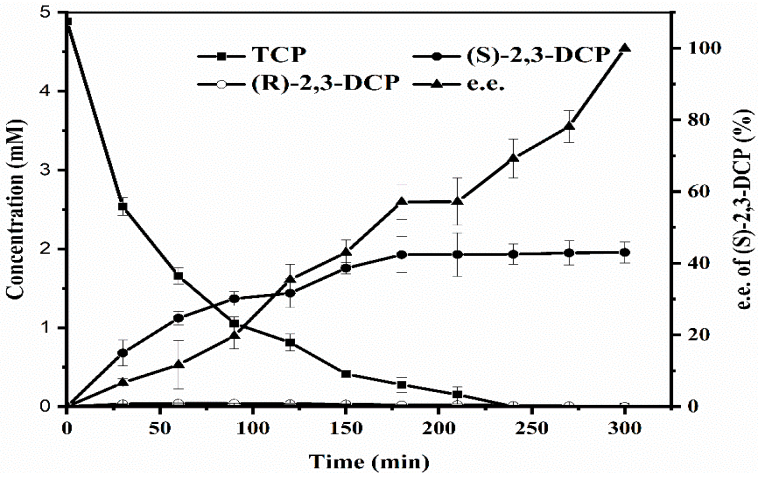

Figure 7. The time course of TCP biodegradation and (*S*)-2,3-DCP synthesis at the optimized condition. The optimized reaction conditions: a reaction mixture of 10 mL borate buffer (0.2 M, pH 8.5) containing 5 mM TCP, 2 mg DhaA31-CLEAs, 0.75 mg HheC-CLEAs, 0.5 g HZD-9 resin, incubated at 37 °C and 180 rpm for 5 h in a screw-capped 20 mL bottle. All experiments were performed in triplicate, the values indicate the mean and the error bars represent standard deviations.

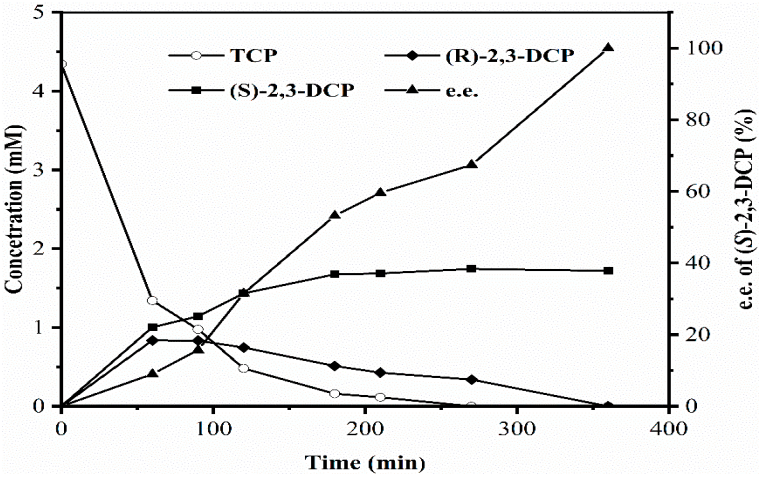

Figure 8. The time course of TCP biodegradation and (*S*)-2,3-DCP synthesis in a scaled-up experiment under the optimized reaction conditions. Using a 500 mL shaking flask, a reaction mixture of 100 mL borate buffer (0.2M, pH 8.5) containing 5 mM TCP, 20 mg DhaA31-CLEAs, 7.5 mg HheC-CLEAs, and 5 g HZD-9 resin, incubated in a rotary shaker at 180 rpm and 37 °C for 6 h. Samples were drawn periodically and prepared for gas chromatography (GC) analysis.

2.2.7. Reusability of DhaA31-CLEAs and HheC-CLEAs

Reusability of enzymes in biocatalysis plays a key role in the economic feasibility of the system. CLEAs can be recycled by centrifugation or filtration. Here we investigated the recyclability of the immobilized enzymes up to six cycles. As shown in Figure 9, the reaction system retained 64% of the initial degradation efficiency. The decrease in enzyme efficiency may be attributed to the recalcitrant nature of TCP, the prolonged reaction time and the negative effect of operation procedures like repetitive washing and centrifugation.

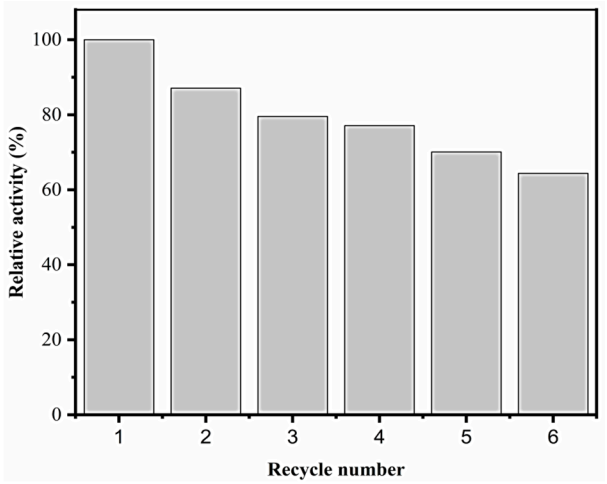

Figure 9. Recycling of immobilized enzymes. The biodegradation of 5 mM 1,2,3-trichloropropane was performed in a borate buffer (0.2 M, pH 8.3) at 37 °C. The activity of the first reaction cycle was set as 100%.

3. Materials and Methods

3.1. Materials

TCP was purchased from Tiexiai (Shanghai) Chengdu Industrial Development Co., Ltd., (*R*,*S*)-2,3-DCP, Pure and racemic ECH were purchased from Alfa Aes ar (Ward Hill, MA). Bovine serum albumin (BSA) was purchased from Sigma, and L-arabinose was purchased from Lancaster Synthesis (Morecambe, UK). HZD-9 resin were provided by Shanghai Huazhen Sci. & Tech. Co., Ltd. (Shanghai, China). Glutaraldehyde, ammonium sulfate, and other materials were purchased from local businesses.

3.2. Protein Expression and Extraction

We apply the recombinant expression of both DhaA31 and the wild-type HheC enzymes in pBAD system as formerly reported by using *Escherichia coli* MC1061 as host cells [19]. Cells were harvested by centrifugation (5000× *g*, 20 min) at 4 °C and were washed once with 10 mM phosphate buffer (pH 8.0). After sonication, enzyme crude extract in 50 mM phosphate buffer (pH 8.0) was obtained by centrifugation (15,000× *g*, 60 min). The protein concentrations were measured using Bradford's method.

3.3. Preparation of CLEAs

DhaA31-CLEAs and HheC-CLEAs were prepared using the procedure reported previously [15] with minor modification. BSA was added slowly and stirred gently as a co-aggregation protein into 1 mL of enzyme crude extract (20 mg/mL) for 15 min. Afterwards, 10 mL of ammonium sulfate (70% saturated, pH 8.0) was added to the enzyme mixture and continuously stirred for 1 h. A certain amount of glutaraldehyde (1 mL, 60 mM in the total mixture volume) was slowly added to the mixture as a cross-linker and was stirred for 4.5 h. The above procedures were all performed in an ice bath. The resulting mixture was centrifuged to collect the aggregates at 4 °C (4000× *g* for 15 min). The aggregates were washed in 20 mL of 50 mM phosphate buffer (pH 7.5) with stirring in an ice bath for 30 min and were later centrifuged. The washing procedure was repeated until no trace of activity was determined in the supernatant. The resulting CLEAs were re-suspended in 2 mL of 50 mM phosphate buffer (pH 8.0) and were stored at 4 °C for further use.

3.4. One-Pot Degradation of TCP in Batch System

The bioremediation of TCP was assayed using 5 mM TCP, 100 mL reaction volume in sodium-borate buffer (0.2 M and pH 8.3) in 500 mL shake flask and incubated at 37 °C and 180 rpm. The reaction initiated by adding 20 mg of DhaA31-CLEAs and 7.5 mg of HheC-CLEAs.

3.5. Reusability of DhaA31-CLEAs and HheC-CLEAs

To evaluate the recyclability of the immobilized enzymes, the CLEAs were used in a reaction with 5 mM substrate under optimal conditions. After the specified reaction time of three hours of which more than 80% of the substrate degraded, the CLEAs were separated by centrifugation, washed with buffer and then re-suspended in a fresh substrate to measure enzyme activity. The residual activity of each enzyme was calculated by taking the enzyme activity of the first cycle as 100%.

3.6. Analytical Methods

The TCP, (*R*,*S*)-2,3-DCP and (*R*,*S*)-ECH were assayed by chiral GC analysis using the chiral column β-dex 225 (30 m x 0.25 mm x 0.25 µm, Supelco) under the following conditions: 100 °C for 6 min, 10 °C/min up to 220 °C, and 15 min at 220 °C; retention times were 12.2 and 12.4 min for S-ECH and (*R*)-ECH respectively, 14.1 min for TCP and 17.2 and 17.4 min for (*S*)-2,3-DCP and (*R*)-2,3-DCP respectively.

Calculations of molar yield (yield) and *e.e.* were performed based on the concentration of TCP and the concentration of (*R*,*S*)-2,3-DCP at different time intervals. The calculation formulae are as follows:

$$yield = \frac{C_{S-DCP}}{C_{0-TCP}} * 100 \quad (1)$$

where C_{S}-$_{DCP}$ and C_{0}-$_{TCP}$ denote the final molar concentration of (*S*)-2,3-DCP and the initial molar concentration of TCP.

$$e.e. = \frac{C_S - C_R}{C_S + C_R} * 100 \quad (2)$$

where C_S and C_R denote the molar concentration of (*S*)-2,3-DCP and (*R*)-2,3-DCP.

4. Conclusions

In this study, resin-integrated one-pot reaction was performed for the synthesis of (*S*)-2,3-DCP from TCP by the combination of CLEAs of both haloalkane dehalogenase mutant DhaA31 and wild-type HheC. The resin-based byproduct removal approach significantly improved the *e.e.* value of (*S*)-2,3-DCP by blocking the circular reaction between ECH and 1,3-DCP. Furthermore, the reaction was systematically optimized with aspects of HZD-9 resin amount, reaction pH and temperature. Under the optimized conditions, The biodegradation of 100 mL, 5 mM TCP in borate buffer (0.2 M and pH 8.5) and enzymatic resolution of (*R*,*S*)-2,3-DCP in the resin-integrated system was successfully carried out in 0.5 L shaking flask at 37 °C and 180 rpm, showing that it can be applied in the treatment of higher volume liquid TCP waste and producing enantiopure (*S*)-2,3-DCP.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4344/10/1/3/s1, Figure S1: Conversion of 1,2,3-trichloropropane(TCP) by haloalkane dehalogenase (DhaA31) and halohydrin dehalognase C (HheC). DhaA31 (step 1), HheC (step 2 and 3, (a) and (b)). Figure S2: The time course of a two stage TCP bioremediation by DhaA31-CLEAs and HheC-CLEAs in n-Heptane-aqueous biphasic system. Reaction conditions: TCP,5 mM; DhaA31-CLEAs, 2 mg; HheC-CLEAs, 2 mg; phosphate buffer (50 mM, pH 8) and phase ratio of 20% (v/v); temperature, 37 °C; incubation time 23 h in a screw-capped 20 mL bottle, Figure S3: The chromatogram from GC analysis of the optimized reaction condition; a) chromatogram at time zero. (b) chromatogram at 2 h and (c) chromatogram at 5 h: an internal standard (ISTD), (S)-2,3-dichloropropane-1-ol ((S)-2,3-DCP). Samples (0.25 mL) drawn from the reaction mixture. The samples were extracted with 0.5 mL of methyl tert-butyl ether (MTBE) containing mesitylene as an internal standard. Gas chromatography (GC) analyses were preformed after the samples were dried with MgSO4 anhydrous. The enantiomeric excess (*e.e.*) of products was determined by chiral GC under the following conditions: 100 °C for 6 min, 10 °C/min up to 220 °C, and 15 min at 220 °C. All separations were carried out using a chiral column β-dex 225 (30 m by 0.25 mm by 0.25 m; Supelco). Figure S4:

The chromatogram from GC analysis of the scaled-up reaction test; (a) chromatogram at 2h (b) chromatogram at 6 h and an internal standard (ISTD), (S)-2,3-dichloropropane-1-ol ((S)-2,3-DCP). Samples (0.25 mL) drawn from the reaction mixture. The samples were extracted with 0.5 mL of methyl tert-butyl ether (MTBE) containing mesitylene as an internal standard. Gas chromatography (GC) analyses were preformed after the samples were dried with MgSO4 anhydrous. The enantiomeric excess (*e.e.*) of products was determined by chiral GC under the following conditions: 100 °C for 6 min, 10 °C/min up to 220 °C, and 15 min at 220 °C. All separations were carried out using a chiral column β-dex 225 (30 m by 0.25 mm by 0.25 m; Supelco).

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References

1. Wang, B.; Chu, K.H. Cometabolic biodegradation of 1,2,3-trichloropropane by propane-oxidizing bacteria. *Chemosphere* **2017**, *168*, 1494–1497. [CrossRef] [PubMed]
2. Dvorak, P.; Bidmanova, S.; Damborsky, J.; Prokop, Z. Immobilized synthetic pathway for biodegradation of toxic recalcitrant pollutant 1,2,3-trichloropropane. *Environ. Sci. Technol.* **2014**, *48*, 6859–6866. [CrossRef] [PubMed]
3. Samin, G.; Janssen, D.B. Transformation and biodegradation of 1,2,3-trichloropropane (TCP). *Environ. Sci. Pollut. Res.* **2012**, *19*, 3067–3078. [CrossRef] [PubMed]
4. Burow, K.R.; Floyd, W.D.; Landon, M.K. Factors affecting 1,2,3-trichloropropane contamination in groundwater in California. *Sci. Total Environ.* **2019**, *672*, 324–334. [CrossRef] [PubMed]
5. Bosma, T.; Damborský, J.; Stucki, G.; Janssen, D.B. Biodegradation of 1,2,3-Trichloropropane through Directed Evolution and Heterologous Expression of a Haloalkane Dehalogenase Gene. *Appl. Environ. Microbiol.* **2002**, *68*, 3582–3587. [CrossRef]
6. Pavlova, M.; Klvana, M.; Prokop, Z.; Chaloupkova, R.; Banas, P.; Otyepka, M.; Wade, R.C.; Tsuda, M.; Nagata, Y.; Damborsky, J. Redesigning dehalogenase access tunnels as a strategy for degrading an anthropogenic substrate. *Nat. Chem. Biol.* **2009**, *5*, 727–733. [CrossRef]
7. Kurumbang, N.P.; Dvorak, P.; Bendl, J.; Brezovsky, J.; Prokop, Z.; Damborsky, J. Computer assisted engineering of the synthetic pathway for biodegradation of a toxic persistent pollutant. *ACS Synth. Biol.* **2014**, *3*, 172–181. [CrossRef]
8. Zou, S.P.; Zheng, Y.G.; Du, E.H.; Hu, Z.C. Enhancement of (*S*)-2,3-dichloro-1-propanol production by recombinant whole-cell biocatalyst in n-heptane-aqueous biphasic system. *J. Biotechnol.* **2014**, *188*, 42–47. [CrossRef] [PubMed]
9. Spelberg, J.H.L.; Vlieg, J.E.T.V.; Bosma, T.; Kellogg, R.M.; Janssen, D.B. A tandem enzyme reaction to produce optically active halohydrins, epoxides and diols. *Tetrahedron Asymmetry* **1999**, *10*, 2863–2870. [CrossRef]
10. van Leeuwen, J.G.; Wijma, H.J.; Floor, R.J.; van der Laan, J.M.; Janssen, D.B. Directed Evolution Strategies for Enantiocomplementary Haloalkane Dehalogenases: From Chemical Waste to Enantiopure Building Blocks. *ChemBioChem* **2012**, *13*, 137–148. [CrossRef]
11. Jin, H.X.; Hu, Z.C.; Liu, Z.Q.; Zheng, Y.G. Nitrite-mediated synthesis of chiral epichlorohydrin using halohydrin dehalogenase from Agrobacterium radiobacter AD1. *Biotechnol. Appl. Biochem.* **2012**, *59*, 170–177. [CrossRef]
12. Majerić Elenkov, M.; Tang, L.; Hauer, B.; Janssen, D.B. Sequential kinetic resolution catalyzed by halohydrin dehalogenase. *Org. Lett.* **2006**, *8*, 4227–4229. [CrossRef]
13. Zou, S.P.; Du, E.H.; Hu, Z.C.; Zheng, Y.G. Enhanced biotransformation of 1,3-dichloro-2-propanol to epichlorohydrin via resin-based in situ product removal process. *Biotechnol. Lett.* **2013**, *35*, 937–942. [CrossRef] [PubMed]
14. Zou, S.P.; Gu, K.; Zheng, Y.G. Covalent immobilization of halohydrin dehalogenase for efficient synthesis of epichlorohydrin in an integrated bioreactor. *Biotechnol. Prog.* **2018**, *34*, 784–792. [CrossRef] [PubMed]

15. Liao, Q.; Du, X.; Jiang, W.; Tong, Y.; Zhao, Z.; Fang, R.; Feng, J.; Tang, L. Cross-linked enzyme aggregates (CLEAs) of halohydrin dehalogenase from Agrobacterium radiobacter AD1reparation, characterization and application as a biocatalyst. *J. Biotechnol.* **2018**, *272–273*, 48–55. [CrossRef] [PubMed]
16. Philips, R.S. Temperature modulation of the stereochemistry of enzymatic catalysisrospects for exploitation. *Trends Biotechnol.* **1996**, *14*, 13–16. [CrossRef]
17. Zhang, Z.J.; Pan, J.; Liu, J.F.; Xu, J.H.; He, Y.C.; Liu, Y.Y. Significant enhancement of (*R*)-mandelic acid production by relieving substrate inhibition of recombinant nitrilase in toluene-water biphasic system. *J. Biotechnol.* **2011**, *152*, 24–29. [CrossRef] [PubMed]
18. Dvorak, P.; Kurumbang, N.P.; Bendl, J.; Brezovsky, J.; Prokop, Z.; Damborsky, J. Maximizing the Efficiency of Multienzyme Process by Stoichiometry Optimization. *ChemBioChem* **2014**, *15*, 1891–1895. [CrossRef] [PubMed]
19. Guo, C.; Chen, Y.; Zheng, Y.; Zhang, W.; Tao, Y.; Feng, J.; Tang, L. Exploring the enantioselective mechanism of halohydrin dehalogenase from Agrobacterium radiobacter AD1 by iterative saturation mutagenesis. *Appl. Environ. Microbiol.* **2015**, *81*, 2919–2926. [CrossRef] [PubMed]