Bienzymatic Cascade Combining a Peroxygenase with an Oxidase for the Synthesis of Aromatic Aldehydes from Benzyl Alcohols

Ma, Yunjian; Li, Zongquan; Zhang, Hao; Wong, Vincent Kam Wai; Hollmann, Frank; Wang, Yonghua

DOI
10.3390/catal13010145

Publication date
2023

Document Version
Final published version

Published in
Catalysts

catalysts

MDPI

Communication

Bienzymatic Cascade Combining a Peroxygenase with an Oxidase for the Synthesis of Aromatic Aldehydes from Benzyl Alcohols

Yunjian Ma [1,2], Zongquan Li [2], Hao Zhang [2], Vincent Kam Wai Wong [1], Frank Hollmann [3,*] and Yonghua Wang [2,4,*]

[1] Neher's Biophysics Laboratory for Innovative Drug Discovery, State Key Laboratory of Quality Research in Chinese Medicine, Macau University of Science and Technology, Taipa, Macau 999078, China
[2] School of Food Science and Engineering, South China University of Technology, Guangzhou 510640, China
[3] Department of Biotechnology, Delft University of Technology, Van der Maasweg 9, 2629 HZ Delft, The Netherlands
[4] Guangdong Youmei Institute of Intelligent Bio-Manufacturing Co., Ltd., Foshan 528200, China
* Correspondence: f.hollmann@tudelft.nl (F.H.); yonghw@scut.edu.cn (Y.W.); Tel.: +31-15-278-1957 (F.H.); +86-20-8711-3842 (Y.W.)

Abstract: Aromatic aldehydes are important aromatic compounds for the flavour and fragrance industry. In this study, a parallel cascade combining aryl alcohol oxidase from *Pleurotus eryngii* (*Pe*AAOx) and unspecific peroxygenase from the basidiomycete *Agrocybe aegerita* (*Aae*UPO) to convert aromatic primary alcohols into high-value aromatic aldehydes is proposed. Key influencing factors in the process of enzyme cascade catalysis, such as enzyme dosage, pH and temperature, were investigated. The universality of *Pe*AAOx coupled with *Aae*UPO cascade catalysis for the synthesis of aromatic aldehyde flavour compounds from aromatic primary alcohols was evaluated. In a partially optimised system (comprising 30 μM *Pe*AAOx, 2 μM *Aae*UPO at pH 7 and 40 °C) up to 84% conversion of 50 mM veratryl alcohol into veratryl aldehyde was achieved in a self-sufficient aerobic reaction. Promising turnover numbers of 2800 and 21,000 for *Pe*AAOx and *Aae*UPO, respectively, point towards practical applicability.

Keywords: aromatic aldehydes; flavour compounds; cascade catalysis; *Pe*AAOx; *Aae*UPO

Citation: Ma, Y.; Li, Z.; Zhang, H.; Wong, V.K.W.; Hollmann, F.; Wang, Y. Bienzymatic Cascade Combining a Peroxygenase with an Oxidase for the Synthesis of Aromatic Aldehydes from Benzyl Alcohols. *Catalysts* **2023**, *13*, 145. https://doi.org/10.3390/catal13010145

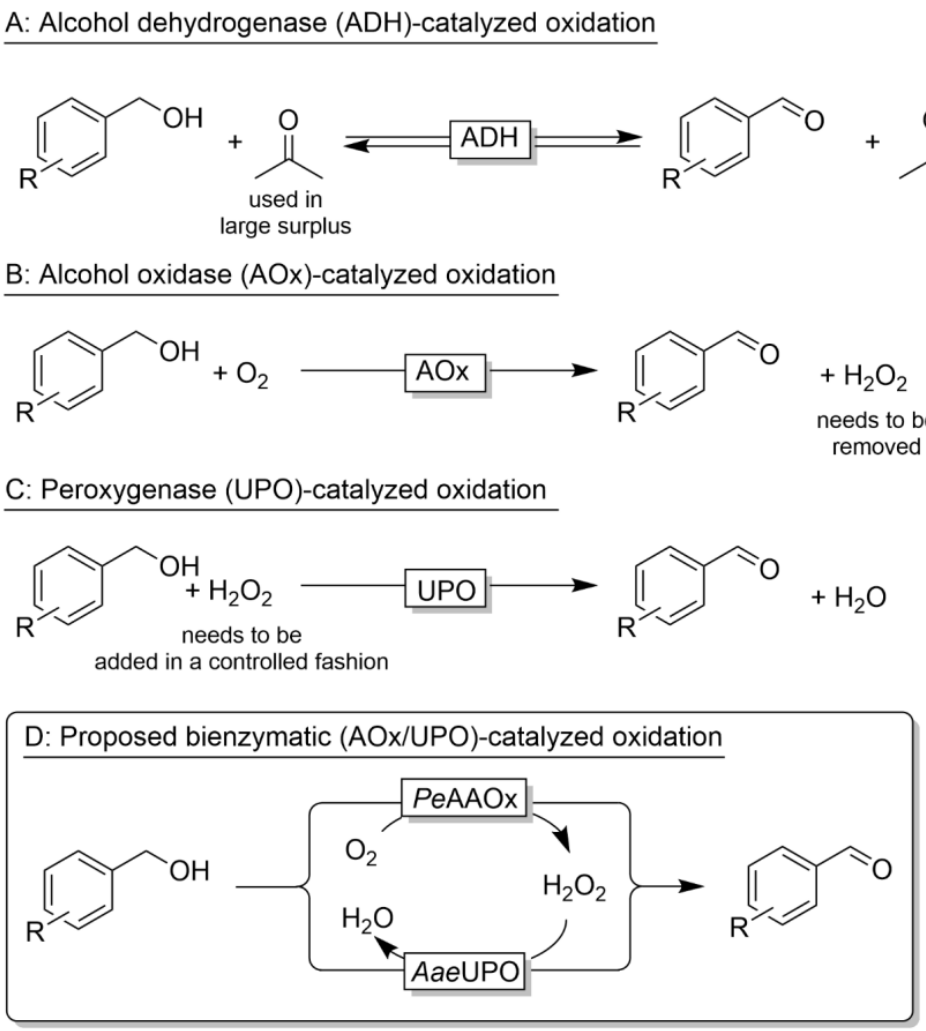
Academic Editors: Chia-Hung Kuo, Chwen-Jen Shieh and Hui-Min David Wang

Received: 19 December 2022
Revised: 4 January 2023
Accepted: 6 January 2023
Published: 8 January 2023

1. Introduction

Aromatic aldehydes are widely found in nature as secondary metabolites, e.g., in plants [1,2]. Commercially, aromatic aldehydes such as vanillin, anisaldehyde or cinnamaldehyde are popular flavour and fragrance ingredients [3–5].

Various chemical synthesis routes for aromatic aldehydes exist [6], but biocatalytic routes are highly desirable, as the products obtained from those are considered as 'natural' [7]. For the transformation of benzylic alcohols into the desired benzaldehyde derivates, a range of biocatalytic methods have been reported (Scheme 1). Alcohol dehydrogenases, for example, catalyse the $NAD(P)^+$-dependent oxidation of benzyl alcohols (Scheme 1A) [8]. Their nicotinamide cofactor-dependency, however, challenges the economic feasibility of these reactions and necessitates further efforts to ensure catalytic use of $NAD(P)^+$ and in situ regeneration. In the simplest scenario, this is achieved by simple administration of a sacrificial hydride acceptor such as acetone, which however complicates the reaction scheme and is less attractive from an environmental point-of-view due to the significant wastes generated by unreacted co-substrate and co-product accumulated. More elegantly, waste-free oxidation using O_2 or H_2O_2 as stoichiometric oxidants would result in environmentally more acceptable reaction schemes.



Scheme 1. Biocatalytic methods for the oxidation of benzylic alcohols. *Pe*AAOx: aryl alcohol oxidase from *Pleurotus eryngii*, *Aae*UPO: (unspecific) peroxygenase from *Agrocybe aegerita*.

Alcohol oxidases catalyse the aerobic oxidation of benzyl alcohols (Scheme 1B) [9]. Particularly, aryl alcohol oxidases (AAOx), have gained considerable interest in recent years [10–17]. An apparent drawback of using AAOx is the stoichiometric formation of H_2O_2 impairing the biocatalyts' robustness. Although H_2O_2 can easily be dimutated by catalase, this approach necessitates a second enzyme, thereby adding to the complexity of the reaction.

Even more recently, so-called unspecific peroxygenases (UPOs) have been reported to mediate the H_2O_2-dependent oxidation of alcohols (Scheme 1C) [18]. Again, H_2O_2 challenges the robustness of the overall reaction, therefore necessitating controlled provision with H_2O_2 [19,20].

The UPO- and AOx-catalysed reactions are co-substrate complementary, i.e., the by-product of the AAOx-catalysed oxidation serves as co-substrate for the UPO reaction. We therefore hypothesised that the combination of AAOx and UPOs may result in a synergistic parallel cascade for the oxidation of benzyl alcohols to the corresponding benzaldehydes (Scheme 1D). Another attractive feature of this system resides in the reduced waste formation of the proposed synergistic reaction scheme. Using an in situ H_2O_2 generation system requires the co-administration of a sacrificial co-substrate and results in the formation of a co-product. This not only negatively influences the environmental footprint of the overall reaction but may also complicate the reaction (e.g., by inhibitory effects of the co-reagents) and downstream processing.

Overall, an aerobic oxidation procedure yielding water as sole by-product was envisioned.

2. Materials and Methods

2.1. Chemical Reagents and Materials

All chemicals were purchased from Sigma-Aldrich (Louis, MO, USA), TCI (Tokyo, Japan), Acros (Morris Plains, NJ, USA) or Aladdin (Shanghai, China) with the highest purity available and used without further treatment.

2.2. Preparation of Enzyme

2.2.1. Preparation of *Aae*UPO

The unspecific peroxygenase from *Agrocybe aegerita* (*Aae*UPO) used in this study was obtained from a previous pilot-scale production of this enzyme [21].

2.2.2. Preparation of *Pe*AAOx

The plasmid pFLAG1-*Pe*AAOx reported previously [22] was kindly provided by Prof. Miguel Alcalde (CSIC, Madrid, Spain).

Cultivation Protocol

pFLAG1-*Pe*AAOx was transformed into *E. coli* BL21 star (DE3). After thermal activation, it was evenly coated with a coating rod on the LB solid medium containing ampicillin, and then it was incubated at 37 °C for 12–18 h at constant temperature until single colonies could be observed, of which one single colony was picked for further cultivation. Precultures of 25 mL LB-medium (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl, 50 mg/L kanamycin) were incubated overnight (12 h, 37 °C, 200 rpm) and used to inoculate the main cultures. The main cultures (500 mL TB-media) were mixed with the inoculum until an optical density of 0.01 was reached. They were then cultivated until an OD_{600} of 0.8 (4 h, 37 °C, 200 rpm) was obtained. Protein overexpression was induced by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG; 1 mM final concentration). The induction time was 4 h and the induction temperature was 37 °C. After induction, cells were harvested by centrifugation (4000 rpm, 20 min, 4 °C). The resultant cell pellet was suspended in 20 mM sodium phosphate buffer, 500 mM NaCl, pH 7.5, and disrupted by sonication on ice. Soluble proteins were separated from cell fragments and insoluble proteins by centrifugation (10,000 rpm, 40 min, 4 °C). The supernatant was filtered through a 0.45 µm cellulose-acetate filter and further processed.

Refolding of PeAAOx from Inclusion Bodies

*Pe*AAOx was purified using an GE Chromatography system (Biorad). Initially, the crude enzyme was injected into a His Prep^{TM} FF16/10 column balanced by washing buffer A (20 mM sodium phosphate buffer, 500 mM NaCl, pH 7.5) at a flow rate of 5 mL min^{-1}. It was then equilibrated by washing buffer A and the binding protein was eluted using the elution buffer B (20 mM sodium phosphate buffer, 500 mM NaCl, 500 mM imidazole, pH 7.5) at a flow rate of 5 mL min^{-1}. Subsequently, the target protein was desalted on the column HiPrepTM 26/10 with the desalting buffer (20 mM sodium phosphate buffer, pH 7.5) at a flow rate of 5 mL min^{-1}. The purified protein was stored at 4 °C. An SDS-PAGE gel of *Pe*AAOx is shown in Figure S1.

2.3. Experimental Set-Up and Operating Conditions

The Agilent 7890B gas chromatography (GC) system (Agilent Technologies, Palo Alto, CA, USA) was used. The KB-FFAP gas chromatography column (30 m × 0.25 mm × 0.25 µm) was used for chromatographic separation. The analysis conditions were as follows: sample volume: 1 µL; solvent: ethyl acetate; injector temperature: 250 °C; split ratio: 30:1; detector: FID; detector temperature: 280 °C. The GC conditions were as follows: initial oven temperature was set at 60–120 °C for 6 min and ramped at 80 °C min^{-1}, and then increased up to 120–230 °C for 8 min and ramped at 20 °C min^{-1}.

Authentic standards of various substances were used to determine the retention time on the GC. Table S1 shows the retention times of various compounds. Standard solutions

of different concentrations were prepared using the above standards; n-dodecane was used as the internal standard. The standard curve is prepared through gas detection for quantitative analysis. In the experiment, the product was not further separated and the conversion is specifically calculated by Formula (1).

$$\text{Conversion\%} = \frac{\text{Product concentration}}{\text{Initial substrate concentration}} \times 100\% \quad (1)$$

2.4. Experimental Procedures

Synthesis of Aromatic Aldehydes via Cascade Reaction of Aromatic Primary Alcohols

Comparison of Catalytic Effects of PeAAOx Coupled with AaeUPO and PeAAOx Alone

(1) PeAAOx coupled with AaeUPO catalytic cascade system experiment

Unless indicated otherwise, sodium phosphate buffer (50 mM, pH 7) was used. The buffer contained *Pe*AAOx (final concentration: 30 μM), *Aae*UPO (final concentration: 2 μM) and the substrate veratryl alcohol (final concentration: 50 mM, pre-dissolved in acetonitrile). The total volume of the reaction was 1 mL. The vessels (4 mL) were placed in self-contained round-hole reaction frames and thermostatted at 40 °C using an oil bath for 6, 12, 24 and 36 h under constant stirring (500 rpm). When the reaction is terminated, the reaction mixture was extracted with an ethyl acetate solution containing 25 mM n-dodecane internal standard, dried with anhydrous sodium sulfate and centrifuged at 12,000 rpm for 3 min. The upper organic phase was then transferred to the chromatographic bottle for GC detection.

(2) PeAAOx catalysis alone experiment

The NaPi buffer (50 mM, pH 7), *Pe*AAOx enzyme solution (final concentration: 32 μM) and veratryl alcohol (final concentration: 50 mM, pre-dissolved in acetonitrile) were added to a 4 mL transparent glass reaction bottle. The total volume of the reaction was 1 mL. The reaction bottle was put on a self-contained round-hole reaction frame and then placed in a constant temperature oil bath for 6, 12, 24 and 36 h at 40 °C and a stirring speed of 500 rpm. The subsequent operation steps are identical to those described in *Pe*AAOx coupled with *Aae*UPO catalytic cascade system experiment.

Effect of PeAAOx Enzyme Dosage on the Oxidation of Veratryl Alcohol

The NaPi buffer (50 mM, pH 7), *Pe*AAOx enzyme solution (final concentrations: 10, 20, 30 and 40 μM, respectively), *Aae*UPO enzyme solution (final concentration: 2 μM) and veratryl alcohol (final concentration: 50 mM, pre-dissolved in acetonitrile) were added to a 4 mL transparent glass reaction flask. The total volume of the reaction was 1 mL. The reaction bottle was put on a self-contained round-hole reaction frame and then placed in a constant temperature oil bath for 3, 6, 9, 12, 24, 36 and 48 h at 30 °C and a stirring speed of 500 rpm. Subsequent operation steps are identical to those described in *Pe*AAOx coupled with *Aae*UPO catalytic cascade system experiment.

Effect of AaeUPO Enzyme Dosage on the Oxidation of Veratryl Alcohol

The NaPi buffer (50 mM, pH 7), *Pe*AAOx enzyme solution (final concentration: 30 μM), *Aae*UPO enzyme solution (final concentrations: 0.5, 1, 2 and 4 μM, respectively) and veratryl alcohol (final concentration: 50 mM, pre-dissolved in acetonitrile) were added to a 4 mL transparent glass reaction flask. The total volume of the reaction was 1 mL. The reaction bottle was put on a self-contained round-hole reaction frame and then placed in a constant temperature oil bath for 4, 8, 12, 24, 36 and 48 h at 30 °C and a stirring speed of 500 rpm. Subsequent operation steps are identical to those described in *Pe*AAOx coupled with *Aae*UPO catalytic cascade system experiment.

Factors Influencing Cascade Catalytic Oxidation of Veratryl Alcohol

(1) Effect of temperature on cascade catalysis

The NaPi buffer (50 mM, pH 7), *Pe*AAOx enzyme solution (final concentration: 30 μM), *Aae*UPO enzyme solution (final concentration: 2 μM) and veratryl alcohol (final concentration: 50 mM, pre-dissolved in acetonitrile) were added to a 4 mL transparent glass reaction bottle. The total volume of the reaction was 1 mL. The reaction bottle was put on a self-contained round-hole reaction frame and then placed in a constant temperature oil bath for 24 h at 25, 30, 35, 40, 45 and 50 °C and a stirring speed of 500 rpm. Subsequent operation steps are identical to those described in *Pe*AAOx coupled with *Aae*UPO catalytic cascade system experiment.

(2) Effect of pH on cascade catalysis

The buffer solution (50 mM, pH 5, 6, 7, 8, 9), *Pe*AAOx enzyme solution (final concentration: 30 μM), *Aae*UPO enzyme solution (final concentration: 2 μM) and then veratryl alcohol (final concentration: 50 mM, pre-dissolved in acetonitrile) were added to a 4 mL transparent glass reaction bottle. The total volume of the reaction was 1 mL. The reaction bottle was put on a self-contained round-hole reaction frame and then placed in a constant temperature oil bath for 24 h at 40 °C and a stirring speed of 500 rpm. Subsequent operation steps are identical to those described in *Pe*AAOx coupled with *Aae*UPO catalytic cascade system experiment.

2.5. Substrate Expansion

The NaPi buffer (50 mM, pH 7), *Pe*AAOx enzyme solution (final concentration: 30 μM), *Aae*UPO enzyme solution (final concentration: 2 μM) and veratryl alcohol, benzyl alcohol, 2-hydroxybenzyl alcohol, cinnamyl alcohol, p-methoxybenzyl alcohol or 4-hydroxy-3-methoxybenzyl alcohol (final concentration: 50 mM, pre-dissolved in acetonitrile) were added to a 4 mL transparent glass reaction flask. The total volume of the reaction was 1 mL. The reaction bottle was put on a self-contained round-hole reaction frame and then placed in a constant temperature oil bath for 24 h at 40 °C and a stirring speed of 500 rpm. Subsequent operation steps are identical to those described in *Pe*AAOx coupled with *Aae*UPO catalytic cascade system experiment.

3. Results and Discussion

In a first set of experiments we compared the catalytic performance of *Pe*AAOx alone with the envisioned bienzymatic cascade (Figure 1).

Pleasingly, the combination of *Pe*AAOx and *Aae*UPO proved to enable faster product formation compared to the single-enzyme catalyzed reaction system (Figure 1). Using *Pe*AAOx as oxidation catalyst alone, the product formation rate was somewhat slower than when using it in combination with *Aae*UPO, which we attribute to a positive effect of the double-catalyst usage postulated (Scheme 1).

Therefore, we further investigated the factors influencing the activity and robustness of the bienzymatic cascade. First, we systematically varied the concentration of either of the two enzymes (Figures 2, 3, S4 and S5). In general, the initial product formation rate of the overall reaction increased with increasing enzyme concentration. This trend was a bit more pronounced when varying the concentration of *Pe*AAOx, which may indicate that this represents the overall rate-limiting step of the cascade reaction. It is also interesting to note that the initial rate did not increase linearly with the *Pe*AAOx concentration. Possibly, at high *Pe*AAOx concentrations, diffusion of O_2 into the aqueous reaction mixture became overall rate-limiting. Finally, it should be noted that the reactions did not reach full conversion. We are currently lacking a plausible explanation for this observation but are convinced that further in-depth characterization of the reaction will reveal the current limitation.

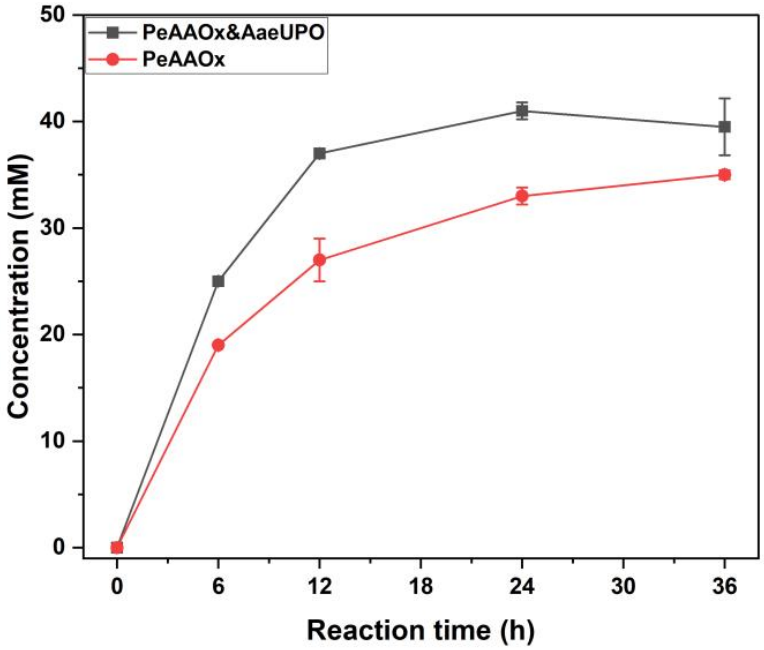


Figure 1. Comparison of catalytic effects of *Pe*AAOx catalysis alone (red) and *Pe*AAOx coupled with *Aae*UPO (black). (Reaction conditions: (1) [*Pe*AAOx] = 32 μM, [veratryl alcohol] = 50 mM (pre-dissolved in acetonitrile), 40 °C, pH 7 and 500 rpm); (2) [*Pe*AAOx] = 30 μM, [*Aae*UPO] = 2 μM, [veratryl alcohol] = 50 mM (pre-dissolved in acetonitrile), 40 °C, pH 7 and 500 rpm).

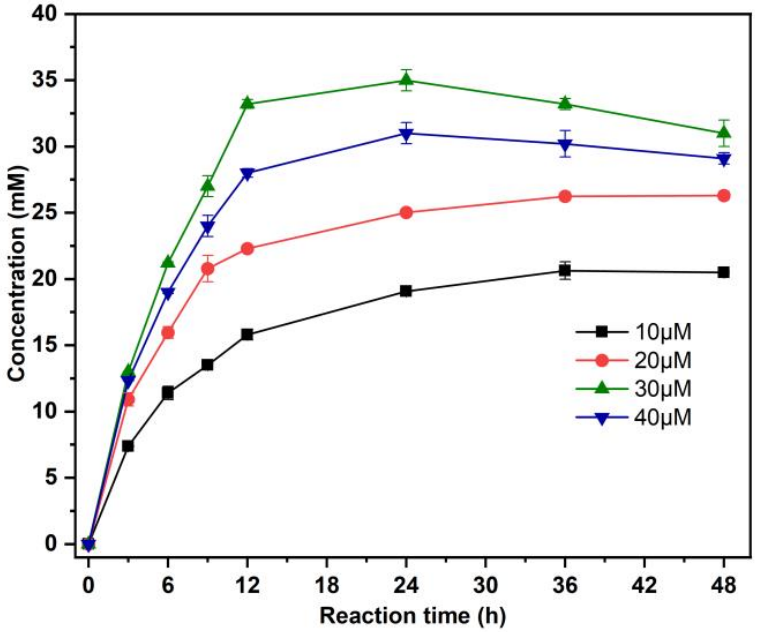


Figure 2. Effects of *Pe*AAOx dosage on the oxidation of veratryl alcohol. (Reaction conditions: [*Pe*AAOx] = 10, 20, 30 and 40 μM, [*Aae*UPO] = 2 μM, [veratryl alcohol] = 50 mM (pre-dissolved in acetonitrile), 30 °C, pH 7 and 500 rpm).

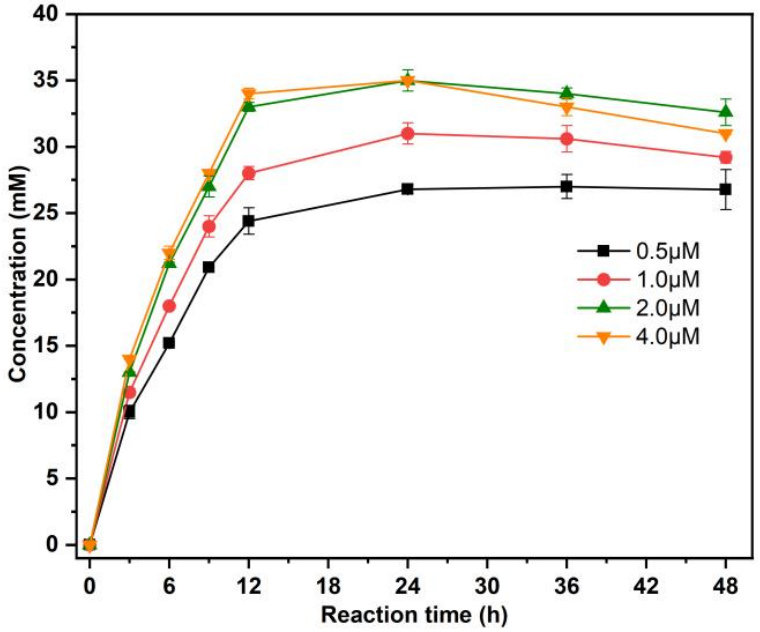


Figure 3. Effect of *Aae*UPO enzyme dosage on the oxidation of veratryl alcohol. (Reaction conditions: [*Pe*AAOx] = 30 μM, [*Aae*UPO] = 0.5, 1.0, 2.0 and 4.0 μM, [veratryl alcohol] = 50 mM (pre-dissolved in acetonitrile), 30 °C, pH 7 and 500 rpm).

Next, the influence of reaction temperature and pH on the overall oxidation rate was examined (Figure 4). Increasing the reaction temperature from 25 °C to 40 °C had only a minor influence on the overall product formation but increasing it to above 40 °C significantly decreased the conversion. We attribute this to the decreasing enzyme stability (Figure S2) and decreasing oxygen solubility at elevated temperatures. The pH profile was relatively broad with considerable activity between pH 5 and pH 8 and culminating at pH 7, which is consistent with the pH optimum of *Pe*AAOx (Figure S3) and *Aae*UPO [23]. Therefore, 40 °C and pH 7 were used for the following experiments.

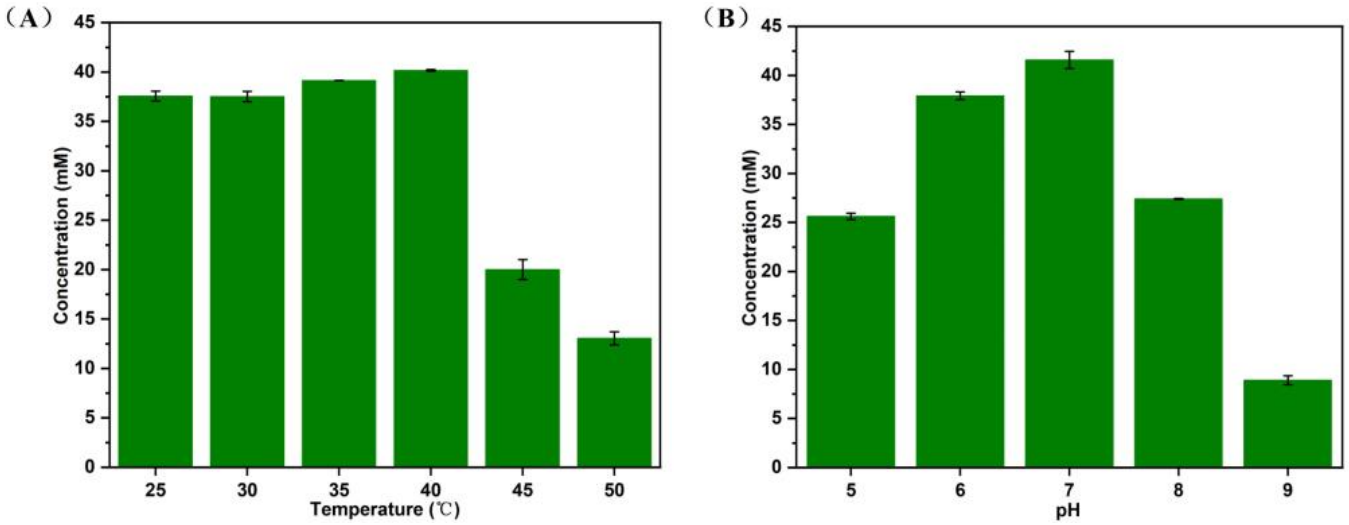
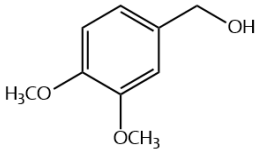
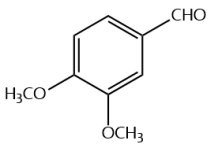
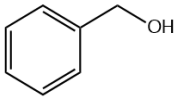
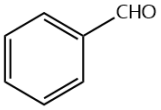
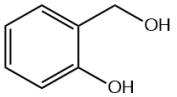
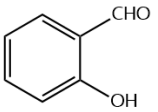
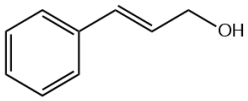
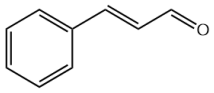
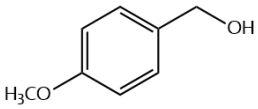
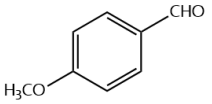
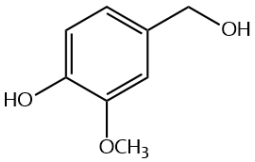
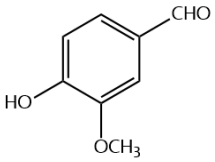


Figure 4. Factors influencing cascade catalytic oxidation of veratryl alcohol. (**A**) Temperature; (**B**) pH. (Reaction conditions: [*Pe*AAOx] = 30 μM, [*Aae*UPO] = 2 μM, [veratryl alcohol] = 50 mM (pre-dissolved in acetonitrile), 500 rpm and 24 h).

To evaluate the synthetic breadth of the proposed bienzymatic alcohol oxidation scheme, we explored the oxidation of further starting materials (Table 1 and Figure S6). A range of ring-substituted benzylic alcohols were converted in acceptable to good yields. Allylic alcohols such as cinnamyl alcohol were also converted, albeit at somewhat lower efficiency. The final product yields, however, correlated only poorly with the reported substrate spectrum of *Pe*AAOx [24]. This may partially be attributed to the initial rate measurements performed previously, which neglect possible inhibitory effects. Moreover, the *Aae*UPO substrate preference may interfere. In any case, a more extensive evaluation of the product scope of the proposed bienzymatic oxidation system will be desirable.

Table 1. Substrate expansion studies [1].

Entry	Substrate	Product	Product Concentration (mM)	Conversion (%)
1			42.03	84.1
2			16.18	36.4
3			5.06	10.1
4			12.27	24.5
5			26.11	52.2
6			16.20	32.4

[1] (Reaction conditions: [*Pe*AAOx] = 30 µM, [*Aae*UPO] = 2 µM, [substrate] = 50 mM (predissolved in acetonitrile), 40 °C, pH 7, 500 rpm, 24 h).

4. Conclusions

In the present study we have established a bienzymatic, parallel cascade combining aryl alcohol oxidases with peroxygenases to selectively oxidise benzylic alcohols into the corresponding aromatic aldehydes. Admittedly, many questions about the efficiency, robustness and scalability of the cascade remain to be answered. But the promising preliminary results obtained so far make us confident that the proposed approach may become a viable route to produce natural flavour compounds.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/catal13010145/s1, Figure S1: SDS-PAGE of *Pe*AAOx; Figure S2: Effect of temperature on *Pe*AAOx enzyme activity (A) and tolerance of *Pe*AAOx to temperature (B); Figure S3: Effect of pH on *Pe*AAOx enzyme activity (A) and tolerance of *Pe*AAOx to pH (B); Figure S4: Gas chromatograms of different *Pe*AAOx enzyme dosages used to catalyze the reaction of aromatic primary alcohols for 24 h; Figure S5: Gas chromatograms of different *Aae*UPO enzyme

dosages used to catalyze the reaction of aromatic primary alcohols for 24 h; Figure S6: The gas chromatogram of the substrate expansion study (the specific peak time is shown in Table S1); Table S1: Retention time of different aromatic primary alcohols/aromatic aldehydes.

Author Contributions: Conceptualization, Y.M. and Z.L.; methodology, Z.L.; software, Z.L.; validation, Y.M., H.Z. and Z.L.; formal analysis, Y.M.; investigation, Z.L.; resources, Z.L.; data curation, Y.M. and Z.L.; writing—original draft preparation, F.H.; writing—review and editing, Y.M.; visualization, Y.M.; supervision, V.K.W.W. and Y.W.; project administration, Y.W.; funding acquisition, Y.M., V.K.W.W. and Y.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Key Realm R&D Program of GuangDong Province (grant number 2022B0202050003), Key Program of Natural Science Foundation of China (grant number 31930084), National Natural Science Foundation of China (grant number 32001633), Guangzhou Science and technology planning project (grant number 202102020370), Macau Young Scholars Program (grant number AM2020024), Dr. Neher's Biophysics Laboratory for Innovative Drug Discovery from the Macau Science and Technology Development Fund (grant number 001/2020/ALC), and FDCT project (grant number 0033/2019/AFJ).

Data Availability Statement: All data are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest. Guangdong Youmei Institute of Intelligent Bio-manufacturing Co., Ltd. provides some experimental instruments for testing during the whole experiment process. The company will not have any conflict of interest.

References

1. Chen, X.-M.; Kobayashi, H.; Sakai, M.; Hirata, H.; Asai, T.; Ohnishi, T.; Baldermann, S.; Watanabe, N. Functional characterization of rose phenylacetaldehyde reductase (PAR), an enzyme involved in the biosynthesis of the scent compound 2-phenylethanol. *J. Plant Physiol.* **2011**, *168*, 88–95. [CrossRef]
2. Torrens-Spence, M.P.; Chiang, Y.-C.; Smith, T.; Vicent, M.A.; Wang, Y.; Weng, J.-K. Structural basis for divergent and convergent evolution of catalytic machineries in plant aromatic amino acid decarboxylase proteins. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 10806–10817. [CrossRef]
3. Panten, J.; Surburg, H. Flavors and Fragrances, 2. Aliphatic Compounds. In *Ullmann's Encyclopedia of Industrial Chemistry*; Wiley-VCH: Weinheim, Germany, 2015. [CrossRef]
4. Panten, J.; Surburg, H. Flavors and Fragrances, 3. Aromatic and Heterocyclic Compounds. In *Ullmann's Encyclopedia of Industrial Chemistry*; Wiley-VCH: Weinheim, Germany, 2015. [CrossRef]
5. Panten, J.; Surburg, H. Flavors and Fragrances, 4. Natural Raw Materials. In *Ullmann's Encyclopedia of Industrial Chemistry*; Wiley-VCH: Weinheim, Germany, 2015. [CrossRef]
6. Punniyamurthy, T.; Velusamy, S.; Iqbal, J. Recent Advances in Transition Metal Catalyzed Oxidation of Organic Substrates with Molecular Oxygen. *Chem. Rev.* **2005**, *105*, 2329–2363. [CrossRef]
7. Fleige, C.; Meyer, F.; Steinbüchel, A. Metabolic Engineering of the Actinomycete Amycolatopsis sp. Strain ATCC 39116 towards Enhanced Production of Natural Vanillin. *Appl. Environ. Microbiol.* **2016**, *82*, 3410–3419. [CrossRef] [PubMed]
8. de Miranda, A.S.; Milagre, C.D.F.; Hollmann, F. Alcohol Dehydrogenases as Catalysts in Organic Synthesis. *Front. Catal.* **2022**, *2*, 900554. [CrossRef]
9. Urlacher, V.B.; Koschorreck, K. Pecularities and applications of aryl-alcohol oxidases from fungi. *Appl. Microbiol. Biotechnol.* **2021**, *105*, 4111–4126. [CrossRef] [PubMed]
10. Lappe, A.; Jankowski, N.; Albrecht, A.; Koschorreck, K. Characterization of a thermotolerant aryl-alcohol oxidase from Moesziomyces antarcticus oxidizing 5-hydroxymethyl-2-furancarboxylic acid. *Appl. Microbiol. Biotechnol.* **2021**, *105*, 8313–8327. [CrossRef] [PubMed]
11. Jankowski, N.; Koschorreck, K.; Urlacher, V.B. Aryl-Alcohol-Oxidase-Mediated Synthesis of Piperonal and Other Valuable Aldehydes. *Adv. Synth. Catal.* **2022**, *364*, 2364–2372. [CrossRef]
12. Jankowski, N.; Urlacher, V.B.; Koschorreck, K. Two adjacent C-terminal mutations enable expression of aryl-alcohol oxidase from Pleurotus eryngii in Pichia pastoris. *Appl. Microbiol. Biotechnol.* **2021**, *105*, 7743–7755. [CrossRef]
13. de Santos, P.G.; Lazaro, S.; Viña-Gonzalez, J.; Hoang, M.D.; Sánchez-Moreno, I.; Glieder, A.; Hollmann, F.; Alcalde, M. Evolved Peroxygenase–Aryl Alcohol Oxidase Fusions for Self-Sufficient Oxyfunctionalization Reactions. *ACS Catal.* **2020**, *10*, 13524–13534. [CrossRef]
14. Viña-Gonzalez, J.; Martinez, A.T.; Guallar, V.; Alcalde, M. Sequential oxidation of 5-hydroxymethylfurfural to furan-2,5-dicarboxylic acid by an evolved aryl-alcohol oxidase. *BBA-Proteins Proteom.* **2020**, *1868*, 140293. [CrossRef]
15. Serrano, A.; Sancho, F.; Viña-González, J.; Carro, J.; Alcalde, M.; Guallar, V.; Martínez, A.T. Switching the substrate preference of fungal aryl-alcohol oxidase: Towards stereoselective oxidation of secondary benzyl alcohols. *Catal. Sci. Technol.* **2019**, *9*, 833–841. [CrossRef]

16. de Almeida, T.P.; van Schie, M.M.C.H.; Ma, A.; Tieves, F.; Younes, S.H.H.; Fernández-Fueyo, E.; Arends, I.W.C.E.; Riul, A.; Hollmann, F. Efficient Aerobic Oxidation of trans-2-Hexen-1-ol using the Aryl Alcohol Oxidase from *Pleurotus eryngii*. *Adv. Synth. Catal.* **2019**, *361*, 2668–2672. [CrossRef]
17. van Schie, M.M.C.H.; de Almeida, T.P.; Laudadio, G.; Tieves, F.; Fernández-Fueyo, E.; Noël, T.; Arends, I.W.C.E.; Hollmann, F. Biocatalytic synthesis of the Green Note trans-2-hexenal in a continuous-flow microreactor. *Beilstein J. Org. Chem.* **2018**, *14*, 697–703. [CrossRef] [PubMed]
18. Hobisch, M.; Holtmann, D.; de Santos, P.G.; Alcalde, M.; Hollmann, F.; Kara, S. Recent developments in the use of peroxygenases—Exploring their high potential in selective oxyfunctionalisations. *Biotechnol. Adv.* **2021**, *51*, 107615. [CrossRef] [PubMed]
19. Burek, B.O.; Bormann, S.; Hollmann, F.; Bloh, J.Z.; Holtmann, D. Hydrogen peroxide driven biocatalysis. *Green Chem.* **2019**, *21*, 3232–3249. [CrossRef]
20. Grogan, G. Hemoprotein Catalyzed Oxygenations: P450s, UPOs, and Progress toward Scalable Reactions. *JACS Au* **2021**, *1*, 1312–1329. [CrossRef]
21. Tonin, F.; Tieves, F.; Willot, S.; van Troost, A.; van Oosten, R.; Breestraat, S.; van Pelt, S.; Alcalde, M.; Hollmann, F. Pilot-Scale Production of Peroxygenase from *Agrocybe aegerita*. *Org. Process. Res. Dev.* **2021**, *25*, 1414–1418. [CrossRef]
22. Ruiz-Dueñas, F.J.; Ferreira, P.; Martínez, M.J.; Martínez, A.T. In vitro activation, purification, and characterization of Escherichia coli expressed aryl-alcohol oxidase, a unique H2O2-producing enzyme. *Protein Expr. Purif.* **2006**, *45*, 191–199. [CrossRef]
23. Ullrich, R.; Nuske, J.; Scheibner, K.; Spantzel, J.; Hofrichter, M. Novel Haloperoxidase from the Agaric Basidiomycete *Agrocybe aegerita* Oxidizes Aryl Alcohols and Aldehydes. *Appl. Environ. Microbiol.* **2004**, *70*, 4575–4581. [CrossRef]
24. Francisco, G.; Angel, T.M.; Maria Jesús, M. Substrate specificity and properties of the aryl-alcohol oxidase from the ligninolytic fungus *Pleurotus eryngii*. *Eur. J. Biochem.* **1992**, *209*, 603–611.