Green Chemistry

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: F. Yang, X. Zhang, F. Li, Z. Wang and L. Wang, *Green Chem.*, 2016, DOI: 10.1039/C6GC00780E.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/greenchem

View Article Online
DOI: 10.1039/C6GC00780E

Journal Name

COMMUNICATION

A lipase-glucose oxidase system for the efficient oxidation of *N*-heteroaromatic compounds and tertiary amines

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

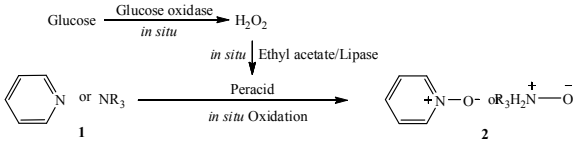
Fengjuan Yang, [a] Xiaowen Zhang, [a] Fengxi Li, [b] Zhi Wang, [a,*] and Lei Wang [a,*]

In this work, a lipase-glucose oxidase system has been designed and proved to be an efficient system for the oxidation of *N*-heteroaromatic compounds and tertiary amines. This dual-enzyme system not only displays environmental friendliness, but also demonstrates the huge potential of industrial applications.

Aliphatic and heterocyclic *N*-oxides (amine *N*-oxides) are important components for their wide usefulness in inorganic and organic chemistry [1-3]. They can be used as protecting groups, auxiliary agents, oxidants, catalysts, surrogates for heterocyclic boronic acids and ligands in metal complexes [4-8]. A variety of catalysts have been developed to prepare these amine *N*-oxides, such as Caro's acid (H_2SO_5), dioxiranes, peracids, activated molecular oxygen/hydrogen peroxide/tert-butylhydroperoxide and magnesium monophthalate [9-15]. However, most of them are expensive, inefficient and toxic. Thus, mining an environmentally clean synthetic method is essential.

Lipase (EC 3.1.1.3) could catalyze the generation of peracids through a perhydrolysis of carboxylic acids or esters in the presence of hydrogen peroxide (H_2O_2). And the formed peroxyacids have been successfully utilized in the epoxidation of alkenes and Baeyer-Villiger reactions [16-20]. In our unpublished research, we found that this method can also be applied for the epoxidation of *N*-heteroaromatic compounds. However, the activated oxygen species such as H_2O_2 or hydroxyl radicals could easily induce the enzyme inactivation and decrease the final oxidation yield. It's well known that glucose oxidase (GOX, EC 1.1.3.4) can generate H_2O_2 in a mild reaction condition with glucose as its substrate. Water and gluconolactone are its side products which is non-toxic and environment friendly [21-22].

Herein, we designed a novel lipase-glucose oxidase system for the efficient oxidation of *N*-heteroaromatic compounds and tertiary amines (Scheme 1). In this system, glucose oxidase, an inexpensive enzyme, was adaped for the *in situ* generation of H_2O_2 to avoid the enzyme inactivation. And then the generated H_2O_2 was used for the lipase-catalyzed synthesis of peracids, which could catalyze the oxidation of *N*-heteroaromatic compounds and tertiary amines. This "feed-on-demand" reaction system is a green and efficient system. Furthermore, this is the first report of a dual-enzyme system contained GOX and lipase.



Scheme 1 Lipase-glucose oxidase system for the oxidation of *N*-heteroaromatic compounds and tertiary amines

Pyridine was used as a model substrate for the oxidation to the corresponding *N*-oxide in the lipase-glucose oxidase system. Lipase B from *Candida antarctica* (CalB) and glucose oxidase from *A. niger* were reported to be the efficient catalysts for the *in situ* generation of peracids and H_2O_2, respectively [23-26]. Therefore, we selected these two enzymes as the catalysts in this work. Initially, the different feeding modes of H_2O_2 in the oxidation of pyridine were investigated and the results were shown in Table 1. Either stepwise or continuous addition of H_2O_2 combined with CalB in this oxidation led to the lower production of the pyridine *N*-oxide (entry 1, 2). The *in situ* generation of H_2O_2 by GOX could improve the efficiency of H_2O_2, avoid the inactivation of lipase, and then higher yield could be obtained (entry 3). It should be noted that in the dual-enzyme catalyzed oxidation of pyridine, the highest yield could be obtained (entry 4) when the oxygen (1 mL/min) was bubbled into the reaction mixture because the activity of GOX would be increased in the oxygen. So, oxygen was used to improve the efficiency of this dual-enzyme system

[a] Key Laboratory of Molecular Enzymology and Engineering of Ministry of Education, School of Life Sciences, Jilin University, 2699 Qianjin street, Changchun, PR China, E-mail: w_lei@jlu.edu.cn, wangzhi@jlu.edu.cn.
[b] School of Life Sciences, Jilin University, 2699 Qianjin street, Changchun, PR China.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

View Article Online
DOI: 10.1039/C6GC00780E

for further investigations. And also, the result of the oxidation catalyzed by chemical catalyst (entry 5) or the blank experiment (entry 6) demonstrated that the lipase-glucose oxidase system is mild, efficient and worthy of further study.

Table 1. The effect of feeding modes of H_2O_2 in the oxidation of pyridine

Entry	Catalyst	Yield
1	CalB+ H_2O_2 (Stepwise) [a]	48.2±1.2
2	CalB+ H_2O_2 (Continuous) [a]	71.3±2.1
3	CalB+GOX (air) [b]	89.1±1.6
4	CalB+GOX (oxygen) [b]	97.6±1.8
5	m-CPBA [c]	53.9±2.7
6	H_2O_2	0

Reaction conditions: [a)] pyridine (1 mmol), Ethyl acetate (EA, 3 mL), CalB (30 U/mL), H_2O_2 (10% aqueous solution, 1.2 mmol), room temperature, 1h; [b)] pyridine (1 mmol), glucose (1.2 mmol), Phosphate buffer (PB, pH 7.0), Ethyl acetate (EA, 2 mL), CalB (30 U/mL), GOX (42 U/mL), room temperature, 1h; [c)] catalyzed by 3-chloroperbenzoic acid (m-CPBA, 1.2 mmol).

The production and consumption of H_2O_2 is a key factor for the dual-enzyme system. Therefore, an optimum operational ratio of GOX and CalB is important in this reaction system. We fixed the concentration of CalB at 30 U/mL and investigated the effects of the ratio of GOX and CalB. The results indicated that the initial formation of H_2O_2 appeared to be the rate-limiting step for this dual-enzyme catalyzed oxidation. As shown in Fig. 1, the yield of pyridine *N*-oxide increased as the ratio of GOX and CalB was increased (1:1-1.4:1). Further increasing the GOX concentration from 1.5:1 to 2.0:1 resulted in gradual decline in the yield of *N*-oxide. This was possibly due to that the accumulation of H_2O_2 in the reaction system could inactivate CalB. We therefore concluded that in this system, the optimal ratio of GOX and CalB was 1.4:1.

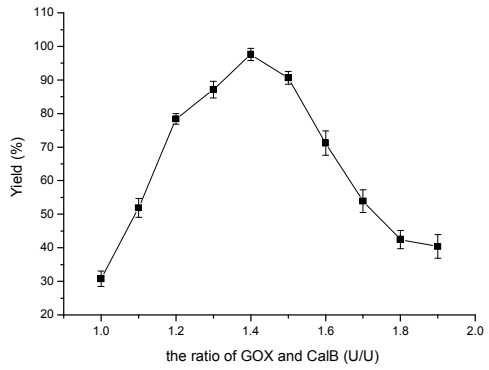


Fig. 1. The effect of the ratio of GOX and CalB in the oxidation of pyridine. Reaction conditions: pyridine (1 mmol), glucose (1.2 mmol), reaction media (3 mL, PB / EA = 1/2), CalB (30 U/mL), oxygen (1mL/min), room temperature, 1h.

The effect of the concentration of enzyme on the yield was investigated when the volume of reaction media was fixed at 3 mL and the ratio of two enzymes (GOX : CalB) was kept constant (1.4:1). It was found that higher concentration of enzymes could enhance the yield of *N*-oxide. But the yield could not be improved with further increasing the concentration of enzymes. Therefore, 30 U/mL of CalB and 42 U/mL of GOX turned out to be sufficient in this dual-enzyme catalyzed oxidation.

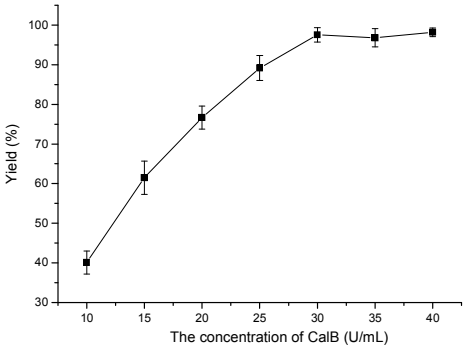


Fig. 2. The effect of the concentration of CalB in the oxidation of pyridine. Reaction conditions: pyridine (1 mmol), glucose (1.2 mmol), reaction media (3 mL, PB/EA=1/2), oxygen (1mL/min), room temperature, GOX:CalB = 1.4:1, 1h.

Reaction medium may seriously affect the catalytic performance and stability of enzyme [27]. It's well known that phosphate buffer is the common solvent for GOX catalyzed reactions [28]. And ethyl acetate can be used as substrate and reaction medium for the lipase-mediated oxidation according to the previous reports [20, 24]. Accordingly, we adapted a bi-phase reaction medium (PB and EA) for this dual-enzyme reaction. The effect of ratio of PB to EA was investigated when the total reaction volume was fixed to 3 mL. As shown in Figure 3, the yield of *N*-oxide exhibited a bell shaped curve with the ratio PB to EA changing, and the optimal ratio was 1:2. The possible explanation may be that the ratio of PB and EA could affect the rate of generation of H_2O_2, and then influence the final yield of *N*-oxide.

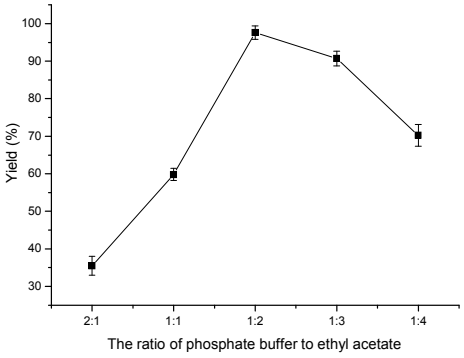


Fig. 3. The effect of reaction media in the oxidation of pyridine. Reaction conditions: pyridine (1 mmol), glucose (1.2 mmol), reaction media (3 mL), CalB (30 U/mL), GOX (42 U/mL), oxygen (1mL/min), room temperature, 1h.

View Article Online
DOI: 10.1039/C6GC00780E

The time course of the enzyme mediated synthesis of pyridine *N*-oxide was illustrated under two type feeding modes of H_2O_2, respectively. As displayed in Fig. 4, the initial reaction rate of the continuous feeding was higher than that of the *in situ* generation mode. After 0.4 h, the oxidation carried out in the dual-enzyme system is significantly faster and affords higher yields than the reaction using the continuous feeding mode of H_2O_2. The oxidation in the dual-enzyme system reached its equilibrium in approximately 1 h with a yield of 97.6% of pyridine *N*-oxide. This interesting observation indicates that the *in situ* generation of H_2O_2 can obviously avoid the enzyme inactivation and increase the final oxidation yield.

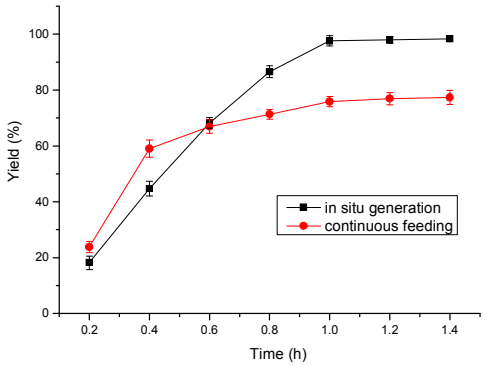


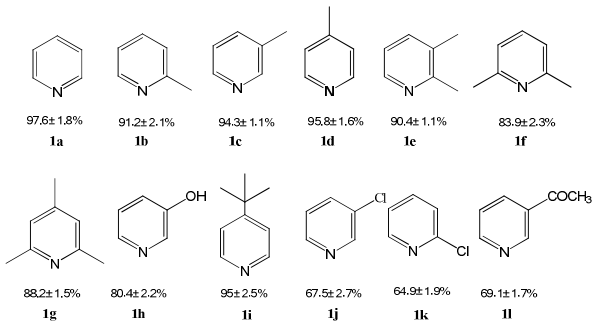
Fig. 4. The time course of the synthesis of pyridine *N*-oxide. Reaction conditions: [a] pyridine (1 mmol), glucose (1.2 mmol), reaction media (3 mL, PB/EA = 1/2), CalB (30 U/mL), GOX (42 U/mL), oxygen (1mL/min), room temperature, 1h; [b] pyridine (1 mmol), EA (3 mL), CalB (30 U/mL), H_2O_2 (10% aqueous solution, 1.2 mmol), room temperature, 1h.

Under the optimal reaction conditions, we scaled up the dual-enzyme system to 100-fold (pyridine (100 mmol), glucose (120 mmol), ethyl acetate (200 mL), phosphate buffer (100 mL), CalB (9000 U), GOX (12600U)). The yield of pyridine *N*-oxide was up to 98.7% after 1h. This result demonstrated that this green method has high potential for industrial production.

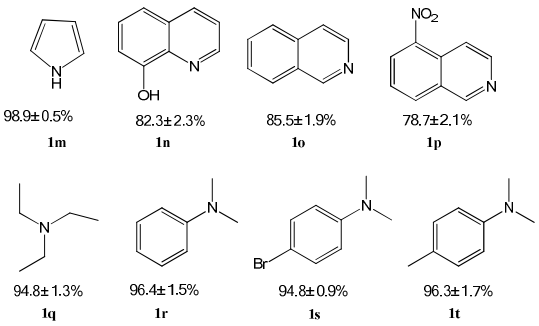
To evaluate the scope and limitation of this method, a wide variety of *N*-heteroaromatic compounds and tertiary amines were used in this oxidation (Table 2). It could be observed from Table 2 that pyridines containing electron donating groups (**1a-1i**) were more active than pyridines bearing electron withdrawing groups (**1j-1l**). And steric effects of the substituents also affected the reaction, because ortho-substituted pyridines resulted in the lower yields. Other *N*-heteroaromatic compounds (**1m-1p**) and tertiary amines (**1q-1t**) can also be oxidized to their corresponding *N*-oxides in good to excellent yields. All the above results indicated that this dual-enzyme system can work well with different *N*-heteroaromatic compounds and tertiary amines in this oxidation.

Table 2. The oxidation of varity N-heteroaromatic compounds and tertiary amines

Pyridine derivatives (1a-1l)



N-heteroaromatic compounds (1m-1p) and tertiary amines (1q-1t)



Reaction conditions: *N*-heteroaromatic compound or tertiary amine (1 mmol), glucose (1.2 mmol), reaction media (3 mL, PB/EA=1/2), CalB (30 U/mL), GOX (42 U/mL), oxygen (1mL/min), room temperature, 1h.

Conclusions

In this work, we reported a novel dual-enzyme system, which can be applied for the oxidation of *N*-heteroaromatic compounds and tertiary amines effectively. Furthermore, this green method can be easily scaled up, and so seems to be a very exciting and promising method which could contribute to the development of new synthetic approache and green technology. However, the reusability of catalyst should be considered in this dual-enzyme system. It's well known that immobilization is a useful tool to improve the reusability and stability of enzyme in modern biotechnology [29-30]. In order to decrease the cost of this dual-enzyme system, a study adopting the technique of co-immobilization is underway and will be reported in due course.

Acknowledgements

We gratefully acknowledge the National Natural Science Foundation of China (no. 21172093), the Natural Science Foundation of Jilin Province of China (no. 20140101141JC) and

View Article Online
DOI: 10.1039/C6GC00780E

Graduate Innovation Fund of Jilin University for the financial support (no. 2015023).

Notes and references

1 K. I. Priyadarshini, M. F. Dennis, M. A. Naylor, M. R. L. Stratford, P. J. Wardman, J. Am. Chem. Soc. 1996, 118, 5648.
2 H. Andersson, R. Olsson and F. Almqvist, Org. Biomol. Chem., 2011, 9, 337.
3 A. V. Malkov, M. Bell, F. Castelluzzo, P. Kocovsky, Org. Lett., 2005, 7, 3219.
4 L. C. Campeau, S. Rousseaux, K. Fagnou, J. Am. Chem. Soc., 2005, 127, 18020;
5 R. Sarma, A. Karmakar and J. B. Baruah, Inorg. Chim. Acta, 2008, 361, 2081.
6 F. F. Bamoharram, M. M. Heravi, M. Roshani, N. Tavakoli, J. Mol. Catal. A: Chem., 2006, 252, 219.
7 V. Vanrheenen, D. Y. Cha, W. M. Hartley, Org. Synth., 1988, 6, 342.
8 J. H. Boyer, Chem. Rev., 1980, 80, 495.
9 G. B. Payne, P. H. Deming and P. H. Williams, J. Org. Chem., 1961, 26, 659.
10 M. Ferrer, F. Sanchez-Baeza and A. Messgure, Tetrahedron, 1997, 53, 15877.
11 L. Rout and T. Punniyamurthy, Adv. Synth. Catal., 2005, 347, 1958.
12 M. R. Prasad, G. Kamalakar, G. Madhavi, S. J. Kulkarni and K. V. Raghavan, Chem. Commun., 2000, 1577.
13 B. M. Choudary, B. Bharathi, C. Venkat Reddy and M. Lakshmi Kantam, Green Chem., 2002, 4, 279.
14 W. Zhao, C. X. Yang, Y. Ding, B. C. Ma, New J. Chem., 2013, 37, 2614.
15 M. Gopiraman, H. Bang, S. G. Babu, K. Wei, R. Karvembu, I. S. Kim, Catal. Sci. Technol., 2014, 4, 2099.
16 R. M. Lau, F. van Rantwijk, K. R. Seddon, R. A. Sheldon, Org. Lett., 2000, 2, 4189.
17 D. Méndez-Sánchez, N. Ríos-Lombardía, V. Gotor, V. Gotor-Fernández, Tetrahedron, 2014, 70, 1144.
18 W. S.D. Silva, A. A.M. Lapis, P. A.Z. Suarez, B. A.D. Neto, J. Mol. Catal. B: Enzym., 2011, 68, 98.
19 S. C. Lemoult, P. F. Richardson, S. M. Roberts, J. Chem. Soc. Perkin Trans. 1, 1995, 89;
20 F. J. Yang, Z. Wang, X. W. Zhang, L. Y. Jiang, Y. Z. Li, L. Wang, ChemCatChem, 2015, 7, 3450.
21 P. C. Pereira, I. W.C.E. Arends, R. A. Sheldon, Process Biochem., 2015, 50, 746.
22 J. A. Miller, L. Alexander, D. I. Mori, A. D. Ryabov, T. J. Collins, New J. Chem., 2013, 37, 3488.
23 V. Skouridou, H. Stamatis, F. N. Kolisis, J. Mol. Catal. B: Enzym., 2003, 21, 67.
24 E. G. Ankudey, H. F. Olivo, T. L. Peeples, Green Chem., 2006, 8, 923.
25 F. H. Zhao, H. Li, Y. J. Jiang, X. C. Wang, X. D. Mu, Green Chem., 2014, 16, 2558.
26 R. D. Milton, F. Giroud, A. E. Thumser, S. D. Minteer, R. C. T. Slade, Phys. Chem. Chem. Phys., 2013, 15, 19371.
27 P. H. Chan, S. W. Tsai, Chem. Eng. Sci., 2016, 139, 41.
28 D. Jung, C. Streb, M. Hartmann, Micropor. and Mesopor. Mat., 2008, 113, 523.
29 R. C. Rodrigues, C. Ortiz, Á. Berenguer-Murcia, R. Torres, R. Fernández-Lafuente, Chem. Soc. Rev., 2013, 42, 6290.
30 O. Barbosa, C. Ortiz, Á. Berenguer-Murcia, R. Torres, R. C. Rodrigues, R. Fernandez-Lafuente, Biotechnol. Adv., 2015, 33, 435.