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Techno-Economic Analysis of Biocatalytic Processes for Production of Alkene Epoxides

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Abstract

A techno-economic analysis of two different bioprocesses was conducted, one for the conversion of propylene to propylene oxide (PO) and other for conversion of styrene to styrene epoxide (SO). The first process was a lipase-mediated chemo-enzymatic reaction, whereas the second one was a one-step enzymatic process using chloroperoxidase. The PO produced through the chemo-enzymatic process is a racemic product, whereas the latter process (based on chloroperoxidase) produces an enantio-pure product. The former process thus falls under the category of high-volume commodity chemical (PO); whereas the latter is a low-volume, high-value product (SO).

A simulation of the process was conducted using the bioprocess engineering software SuperPro Designer v6.0 (Intelligen, Inc., Scotch Plains, NJ) to determine the economic feasibility of the process. The purpose of the exercise was to compare biocatalytic processes with existing chemical processes for production of alkene expoxides. The results show that further improvements are needed in improving biocatalyst stability to make these bioprocesses competitive with chemical processes.

Index Entries: Bioprocess; chloroperoxidase; economics; enzymatic; epoxides; lipase.

Introduction

Biocatalytic production of chemicals and intermediates has been studied at the laboratory level for several years; however, few industrial-scale processes have been commercialized. Commercial production of commodity organic chemicals using enzymatic catalysts has been almost nonexistent. However, with the increased use of biocatalytic processes in low-volume high-value chemicals such as pharmaceutical intermediates, newer technologies improving the activity and stability of enzyme biocatalysts *(1,2)* for industrial applications have emerged. Some of these include improved immobilization techniques *(3)*, stabilization through lyophilization with lyoprotectants *(4)*, salts *(5)*, or addition of surfactants *(6,7)*, chemical modification with amphiphilic or hydrophobic polymers *(8,9)*.

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Epoxidation of alkenes is of significant industrial interest because of production of a range of products from the epoxides including glycols, polymers such as polyols, plasticizers, and stabilizers for polyurethane manufacturing, pharmaceuticals, agrochemicals, and so on. Biocatalytic epoxidation of alkenes is possible through microbial or enzymatic processes. A microbial process is preferred when cofactor regeneration is necessary, as in monooxygenase-based processes *(10)*. Alternately, an enzymatic process using fatty acids as peroxidation cosubstrates *(11–13)* may be used. Epoxidation of terminal alkenes with carbon atoms ranging from 8 to 16 carbon atoms as well as some cycloalkenes has been demonstrated *(11)*. A second enzymatic route is through the use of haloperoxidases such as chloroperoxidase (CPO) from *Caldariomyces fumago*. This conversion results in epoxides with enantiomeric excess as high as 95%, although yields are generally moderate *(14)*.

Propylene oxide (PO) is one of the largest volume epoxides produced worldwide using chemical routes. A bioprocess route has potential to increase the energy efficiency of producing this commodity chemical. Two biological routes have previously been proposed for production of PO. These include a four-step Cetus process based on corn liquor fermentation and a second route based on methane monooxygenase (MMO) *(15)*. A technical and economic analysis and comparison of various bioreactor types for the latter process was also reported *(16)*. A third route being proposed here is based on a two-step chemo-enzymatic process using lipase as catalyst. A process model based on the two-step reaction was developed for industrial production of PO and an order of magnitude economic analysis was conducted.

Similarly, the epoxidation of styrene through chloroperoxidase was used as a model for developing the process model, followed by the economic feasibility analysis of the process. It should be noted that the chloroperoxidase-based process produces styrene oxide (SO) at high enantiomeric excess, whereas the (PO) is a racemic product. In the latter, whereas the first step is enzymatic, the second step, which produces PO is a chemical step. To develop a process for high-volume commodity chemicals such as PO, enantiopurity is not considered necessary.

Results and Discussion

Propylene Oxide Process Description

The production of PO is based on a two-step chemo-enzymatic reaction using lipase, a fatty acid, and hydrogen peroxide. The two reaction steps are given below:

$$H_2O_2 + \text{Fatty acid (using lipase as catalyst)} \rightarrow \text{Peroxy acid} + H_2O \quad (1)$$

$$\text{Propylene} + \text{Peroxy acid} \rightarrow \text{PO} + \text{Fatty acid (recycled)} \quad (2)$$

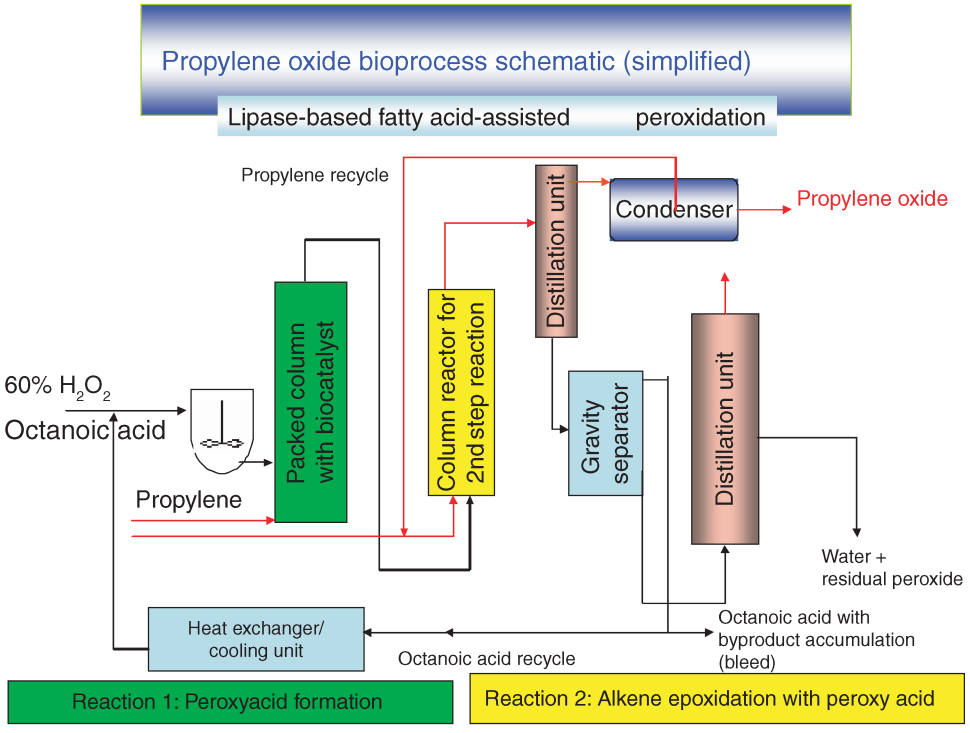

Fig. 1. Schematic for conversion of propylene to propylene oxide using a lipase-based chemo-enzymatic peroxidation process.

A chemical route based on peroxy acid as an oxidant has been reported previously. In such a process, acetic acid is suggested as the fatty acid for use. However, use of a low-molecular weight fatty acid interferes with subsequent product separation. Thus, use of higher molecular weight fatty acids is preferable. The enzymatic route allows use of higher fatty acids of up to 20 carbon atoms with very good conversion to peroxy acid *(13)*. Although the reactivity of these higher fatty acids with propylene has not been demonstrated, they have been shown to be effective in epoxidizing terminal alkenes with 8–16 carbon atoms *(11)*. A few assumptions have been made in developing the process model, with the reactivity of specifically, peroxyoctanoic acid with propylene, being one of them. Other assumptions are described later.

The first step in the PO process is conversion of a fatty acid, such as octanoic acid to octane peroxy acid using lipase and H_2O_2. The peroxy acid is then used as a chemical oxidant to oxidize propylene-to-propylene oxide in the second step. Such a process is applicable to epoxidation of unsaturated plant oils as well *(17,18)*. A schematic of the process for production of PO is shown in Fig. 1. In this preliminary design developed for the process, octanoic acid and 60% hydrogen peroxide are first mixed in a blending tank. The solubility data on hydrogen peroxide solubility in octanoic acid is not available, so an approximation based on the solubility

data of water in octanoic acid (3.88% at 14.4°C) *(19)* was used. A maximum solubility of 3% was assumed for 60% hydrogen peroxide in octanoic acid at 25°C. The octanoic acid solution containing the dissolved peroxide is then passed through a packed column containing the appropriate biocatalyst. In this study, the biocatalyst used was an immobilized lipase (Novozym-435; Novozymes, Franklinton, NC), for which kinetic data is available for the reaction of epoxidation of unsaturated fatty acids. The kinetic rate constants for alkene epoxidation is not available, although the conversion data is available. However, because rate data is required for determining process conversion, an approximation was used.

Deactivation of the lipase biocatalyst is included as a third reaction (disappearance of the biocatalyst). The rate of deactivation is used from a study by Hilker *(17)*, which gives kinetic constants at various temperatures. The reactor 1 was designed to operate at 40°C and the rate of deactivation of the biocatalyst used was 0.036/min in the organic phase saturated with 60% H_2O_2. The propylene gas is flown cocurrent (upward) and recycled to achieve higher conversion. The operating temperature in the reactor is 40°C, allowing the product PO to leave the reactor as a gas along with the unconverted reactant, propylene. This gas mixture is sent to a condenser to remove product PO from propylene. The liquid mixture from the reactor (containing water and residual hydrogen peroxide, dissolved propylene, PO, and octanoic acid) is passed through a gravity separator to remove any aqueous phase carried over with the organic phase. Any aqueous phase collecting in the separator is flash distilled to remove dissolved PO. The organic phase is passed through a distillation tower to remove water (any byproducts formed, such as propylene glycol may also be removed in this manner, although byproduct formation is assumed to be zero in this simulation). The bottoms stream from the distillation tower contains octanoic acid and octane peroxy acid. This stream is recycled to the reactor 1. A recycle loop is also provided for propylene from the condenser outlet as well as the distillation unit.

To enable process design and economic analysis of the process, a simulation of the process was conducted (using SuperPro Designer). A process flow diagram is given in Fig. 2. It should be noted that two reactors in series are used for the two serial steps, wherein the first one is simulation of a packed bed reactor and the second one is a plug flow reactor. Several assumptions were made in designing the process and in its simulation. The major assumptions are listed below:

1. Kinetic data from lauric/stearic acid peroxidation *(17)* is applicable for first step.
2. Kinetic data from oleic acid/alkene epoxidation *(17)* is applicable for second step.
3. Aqueous solubility (water + H_2O_2) in octanoic acid of 3% was assumed.

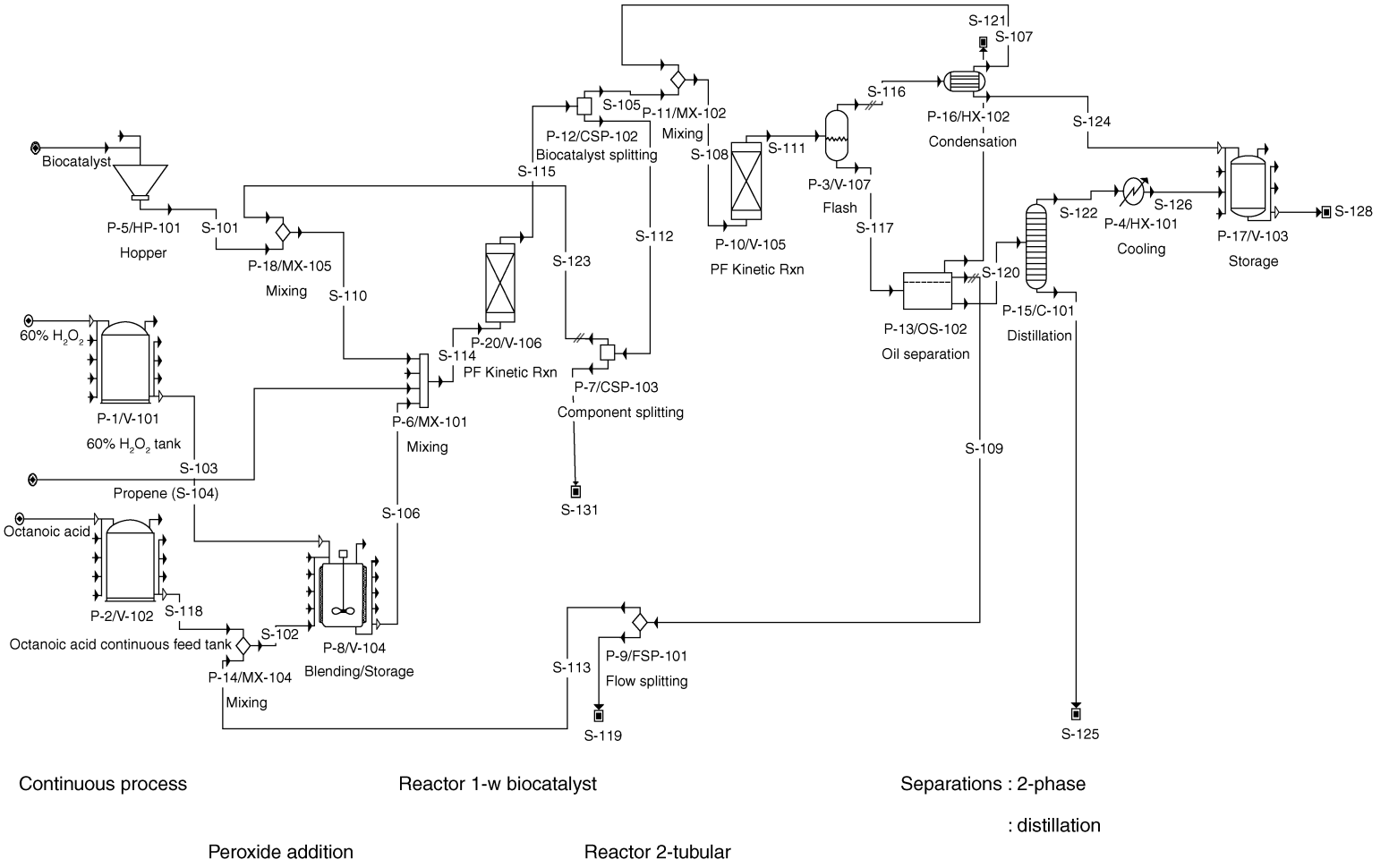

Fig. 2. Process flow diagram for propylene oxide production using a lipase-based chemo-enzymatic peroxidation process.

Executive summary (2005 prices)

Total capital investment	169,071,000	$
Capital investment charged to this project	169,071,000	4
Operating cost	823,708,000	$/yr
Production rate	199,160,052.85	kg of MP/yr
Unit production cost	4.14	$/kg of MP
Total revenues	290,741,000	$/yr

Annual operating cost (2005 prices) - Process summary

Cost item	$	%
Raw materials	752,183,000	91.32
Labor-dependent	6,441,000	0.78
Facility-dependent	17,187,000	2.09
Laboratory/QC/QA	966,000	0.12
Consumables	0	0
Waste treatment/disposal	1,424,000	0.17
Utilities	45,508,000	5.52
Transportation	0	0
Miscellaneous	0	0
Advertising/selling	0	0
Running royalties	0	0
Failed product disposal	0	0
Total	823,708,000	100

Raw materials cost - Process summary

Bulk raw material	Unit cost ($/kg)	Annual amount (kg)	Annual cost ($)	%
60% H_2O_2	0.396	196,051,680	77,715,000	10.33
Octanoic acid	1,320	4,356,000	5,750,000	0.76
Propene	0.677	145,569,600	98,478,000	13.09
Biocatalyst	900	633,600	570240,000	75.81
Total		346,610,880	752,183,000	100

Fig. 3. Results from preliminary economic analysis of an enzymatic propylene oxide production process.

4. Separation of bulk aqueous phase from octanoic acid phase can be achieved by gravity separation.
5. No royalty cost was assumed in economic analysis.
6. Waste disposal cost was assumed to be negligible.
7. Cost of propylene, hydrogen peroxide, lipase enzyme, and propylene oxide was assumed to be $0.67, 0.66, 900, and 1.58/kg, respectively. This is based on pricing obtained from commercial vendors and Chemical Market Reporter magazine (July 5–12 2004).
8. No byproducts were assumed to be formed in this analysis.
9. No degradation of the peroxy acid occurs in the distillation tower.
10. The biocatalyst deactivation rate (essentially owing to peroxide) obtained for octanoic acid–propylene-Novozym 435 system was presumed to be similar to that reported by Hilker et al. *(17)* for toluene–oleic acid-Novozym 435 system.

The assumptions are presumed suitable for an order-of-magnitude analysis and it should be made clear that many of these assumptions may have to be revised if a more detailed process simulation is to be undertaken. The results of the analysis are shown in Fig. 3. The results indicate

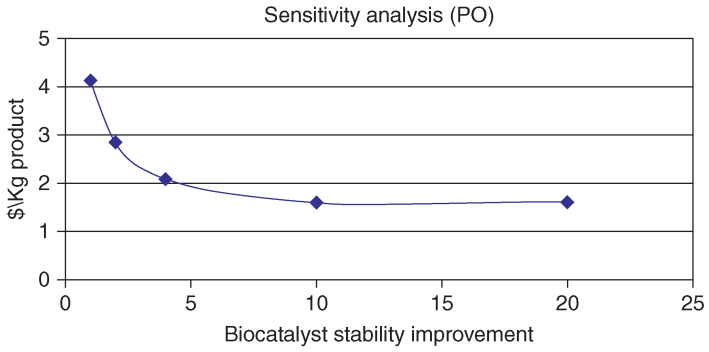

Fig. 4. Sensitivity analysis for production of propylene oxide with respect to enzyme stability. An order of magnitude improvement in enzyme stability (reduction in deactivation constant) is needed for the process to produce PO at a cost comparable with current chemical commercial process (PO-Styrene process). The baseline enzyme stability used in the study was for Novozym 435, which is a polyvinyl acetate-immobilized form of the lipase enzyme.

that the process is uneconomical considering the price of biocatalyst used ($900/Kg, Novozym 435). The cost of production of PO was determined to be about $4.14/kg, which is 2.6-fold higher than the current selling price of PO ($1.58/kg). The cost of raw materials (including enzyme) is 91% of the cost of production. Furthermore, the enzyme costs amount to about 75% of the total raw material cost. Thus, improvements are needed either in the biocatalyst activity, stability, or its purchase cost.

A sensitivity analysis was conducted based on the cost and the stability of the enzyme. The cost of the product PO was found to be controlled largely by lipase enzyme and its stability. This cost can potentially be reduced as the demand for the enzyme rises. For the biocatalyst, Novozym 435, used in this analysis, the cost has to drop to $45/kg for the process to produce PO economically, based on this order of magnitude analysis. The sensitivity analysis was done with respect to biocatalyst stability using the price of $900 to find out how stability improvement would affect production cost. The result is given in Fig. 4. The plot shows that a 10- to 20-fold improvement in stability is necessary to bring the production cost near the current product-selling price. It should be noted that the price listed ($900/kg) is for the biocatalyst itself and not the cost for producing a kilogram of product. Second, the price was obtained from enzyme manufacturers that sell the enzymes in bulk. Thus, the prices are realistic prices at this time for the current demand. The prices will certainly decrease as demand increases and technology advances.

A comparison with previously suggested biochemical as well as chemical routes was conducted. Based on the production costs reported previously *(15)*, the three chemical routes, chlorohydrin, *t*-butyl alcohol, and the styrene byproduct routes resulted in PO production cost of $1.548, 1.219, and 0.87/kg of PO, respectively, calculated for 2005 using chemical

plant cost index. The Cetus process gave a production cost of $1.89, whereas the Exxon MMO process gave a cost of $1.14/kg of PO. Although these production costs are relatively low compared with the cost of production using the lipase-based chemo-enzymatic process ($4.14/kg of PO), it should be stressed that in the bioprocess calculations for the Cetus and MMO process, the cost of biocatalyst was not appropriately accounted for. For example, the Cetus process uses three enzymes, glucose isomerase, chloroperoxidase, and epoxide hydrolase as well as a palladium catalyst, and the total catalyst cost (for all four) was assumed to be only 15% of propylene cost. In the MMO process, the cost of the microbial MMO catalyst was about 20% of the propylene cost. A more detailed analysis *(16)* for the MMO process reported a cost of $26.6/kg of PO using a conventional reactor and a cost of $11/kg with an improved reactor (granular-activated carbon-fluidized bed reactor).

SO Production Process

A similar order of magnitude process analysis was also conducted for the production of SO. The design of this process was based on experimental system developed elsewhere *(20,21)*. A batch process was designed for production of 200 *t*/yr of SO. The flow sheet is given in Fig. 5. It consists of a two-phase bioreactor consisting of 1% styrene as the organic phase and 99% aqueous phase containing the enzyme, chloroperoxidase. The organic substrate is emulsified in the aqueous buffer using the surfactant aerosol-OT. Such a system was reported to increase the total turnover number (TTN) by one order of magnitude *(21)*. A process based on the 2-phase system without the surfactant was also evaluated. The process was conducted in batch with a 16 h batch and fed-batch addition of the enzyme, hydrogen peroxide, and the substrate styrene (based on the rate of conversion). The design was based on production of 600 kg of SO per batch, separated through vacuum distillation. The following assumptions were made in the analysis. The first four assumptions are based on experimental work reported elsewhere *(21)*.

1. Assume stoichiometric conversion to obtain 1.3 g/L/h productivity of SO.
2. Assume TTN of 13,000, equivalent to 27.0 mg/L/h of enzyme use.
3. Total volume of aqueous phase approx 28,500 L; 267.0 kg of styrene (added at 1% [w/w]).
4. Provide hydrogen peroxide in stoichiometric amounts, at rate 7.2 mmoles/L/h.
5. Use oil–water separator for phase separation and assume 99% SO recovery from the aqueous–organic mixture.
6. Use batch distillation at 0.01 bar and 75°C *(22)* to achieve 90.3% SO with 9.7% styrene in product, and recover balance styrene approx 224.0 kg for recycle (*Note*: This is shown as revenue stream here, along with SO product, but will be recycled).

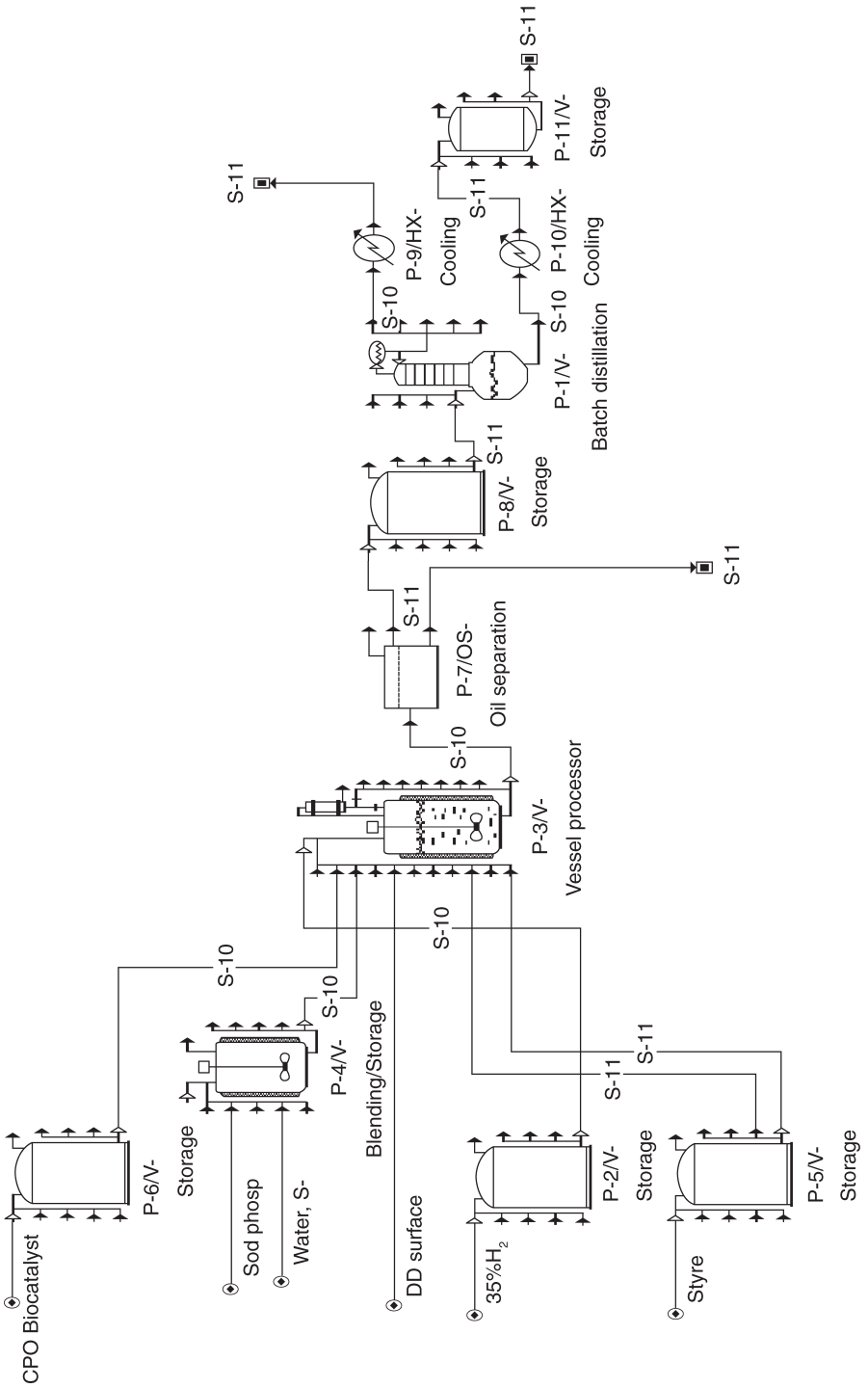

Fig. 5. Flow sheet for production of StyOx in a batch process using SuperPro Designer.

Executive summary (2005 prices)

Total capital investment	31,721,000 $
Capital investment charged to this project	31,721,000 $
Operating cost	221,672,000 $/yr
Production rate	192,203.78 kg of MP/yr
Unit production cost	1,153.32 $/kg of MP
Total revenues	4,872,000 $/yr

Annual operating cost (2005 prices) - Process summary

Cost item	$	%
Raw materials	213,857,000	96.47
Labor-dependent	4,952,000	2.23
Facility-dependent	2,116,000	0.95
Laboratory/QC/QA	743,000	0.34
Consumables	0	0
Waste treatment/disposal	0	0
Utilities	3,000	0
Transportation	0	0
Miscellaneous	0	0
Advertising/selling	0	0
Running royalties	0	0
Failed product disposal	0	0
Total	221,672,000	100

Raw materials cost - Process summary

Bulk raw material	Unit cost ($/kg)	Annual amount (kg)	Annual cost ($)	%
Sodium phosphate	0.100	117,785	12,000	0.01
Water	0.001	9,494,579	9,000	0
Hydroperoxide	0.660	57,749	38,000	0.02
Styrene	1.210	264,762	320,000	0.15
Biocatalyst	50,000	4,261	213,036,000	99.62
Dioctyl sulfosu	5	88,330	442,000	0.21
Total		10,027,465	213,857,000	100

Fig. 6. Results from preliminary economic analysis of an enzymatic StyOx production process.

7. A yield of 100% is assumed and no byproducts are assumed to be produced.
8. Biocatalyst (CPO) price was initially assumed to be about $5/mg.

The enzyme price was obtained from vendors who supply the purified enzyme, but have only laboratory-scale operations for production of CPO (price between $5 and $16/mg). In a recent review *(23)*, the price of CPO obtained from Chirazym was $5/mg. Although this is not an appropriate price for evaluating a commercial application, a better estimate for this fungal enzyme is not available. Although research on development of improved methods to produce CPO are under way *(23,24–26)*, a significantly cheaper way to produce this enzyme has not been reported. Assuming a 100-fold decrease in the price of the enzyme compared with current methods of production gives a price of CPO = $0.05/mg. Thus, an approximate estimate of $50,000/kg was used in the analysis.

The results of the economic analysis are shown in Fig. 6. The results show that the biocatalyst cost (using a price of $50,000/kg) contributes to

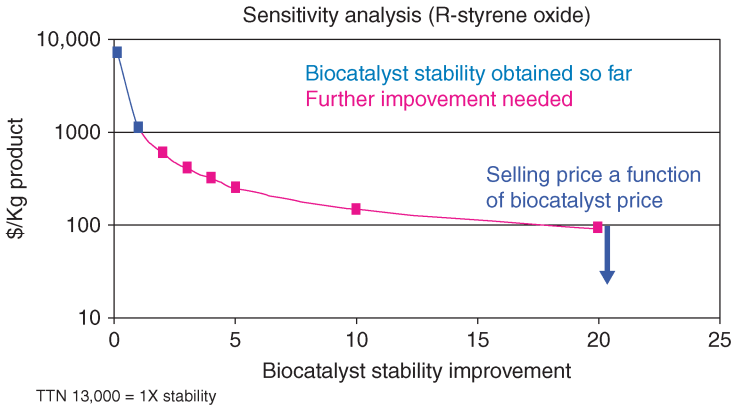

Fig. 7. Sensitivity analysis for production of styrene epoxide with respect to enzyme stability. The stabilization procedure developed at University of California, Berkeley accounts for a ninefold decrease in cost compared with existing data.

more than 95% of the production cost. Thus, the biocatalyst cost is the major contributor to the product price. Industrial enzymes are now available at much reduced prices, as genetically engineered strains have been developed to produce the enzymes. Enzymes such as amyloglucosidase and cellulases are available in the range of few hundred dollars per kilogram from Genencor (Palo Alto, CA), Novozymes, and other bulk enzyme manufacturers. However, it should be noted that the source of CPO is the fungus, *C. fumago*, and not all fungal enzymes can be expressed at high levels. Enzymes such as hemicellulases and cellulases from fungal sources have been produced in *Aspergillus niger* and *Trichoderma reesei* at low production costs, so a method for producing CPO may be possible. Recently, expression of CPO was reported in *A. niger*, although the level of production was relatively low *(25)*.

Alternate means to improve the process economics is through activity and stability improvement *(9,25,27,28)*. A sensitivity analysis (Fig. 7) was conducted to determine what other parameters would be important besides the biocatalyst price. Both activity and stability were altered in the process model and it was found that the impact of the biocatalyst stability was much higher. This was because the biocatalyst loses activity rapidly in the presence of the peroxide. Therefore, even if its initial activity is high, the conversion remains low, unless the activity is maintained for a sufficient time. In Fig. 7, the production cost based on a process without the use of a surfactant *(29)* was calculated. This was determined to be more than $9000/kg of SO.

Based on the improvements reported recently in this system, (increase in TTN from 1500 *[29]* to 13,000 with use of a surfactant-based process), the cost of StyOx drops to about $1000/kg. A further 10-fold improvement in biocatalyst stability is needed to enable reduction of production cost to the $100 range. The selling price of enantiopure SO is estimated to be about

$25/kg. Thus, additional reductions in the cost of the enzyme are necessary to make the process economically feasible. An alternate bioprocess to make SO has been reported using a microbial catalyst *(10)*; however, no economic feasibility studies have been reported for the process.

Conclusions

The preliminary process design for production of PO and SO based on enzymatic processes were conducted. The results indicate that the cost of the enzyme contributes significantly to the production cost and can be as high as 90–95% of the total cost, using current enzyme price. In case of the PO process, which uses an immobilized lipase enzyme available commercially, the cost is still dominated by the biocatalyst cost. This was found to be a result of the instability of the enzyme in presence of hydrogen peroxide, which is a cosubstrate. Thus, improvements are needed in stabilizing the lipase enzyme against hydrogen peroxide deactivation in peroxidation reactions. The feasibility of production of enantiopure SO was similarly found to be controlled by cost of the enzyme, chloroperoxidase. In this case, although the product is of higher value, the economic feasibility was not better because of the higher expected cost of producing the fungal enzyme. Thus, the primary need for demonstrating process feasibility is being able to produce the enzyme CPO at a reduced cost. Furthermore, the stability of the enzyme was important for this enzyme as well and at least an order of magnitude improvement in peroxide stability is needed in each of these enzymes to improve economical feasibility of the process.

Acknowledgments

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References

1. Adamczak, M. and Krishna, S. H. (2004), *Food Technol. Biotechnol.* **42,** 251–264.
2. Gupta, M. N. and Roy, I. (2004), *Eur. J. Biochem.* **271,** 2575–2583.
3. D'Souza, S. F. (1999), *Curr. Sci.* **77,** 69–79.
4. Dai, L. Z. and Klibanov, A. M. (1999), *Proc. Natl. Acad. Sci. USA* **96,** 9475–9478.
5. Morgan, J. A. and Clark, D. S. (2004), *Biotechnol. Bioeng.* **85,** 456–459.
6. Sawae, H., Sakoguchi, A., Nakashio, F., and Goto, M. (2002), *J. Chem. Eng. Japan* **35,** 677–680.
7. Song, B. D., Ding, H., Wu, J. C., Hayashi, Y., Talukder, M., and Wang, S. C. (2003), *Chin. J. Chem. Eng.* **11,** 601–603.
8. Mine, Y., Fukunaga, K., Yoshimoto, M., Nakao, K., and Sugimura, Y. (2001), *J. Biosci. Bioeng.* **92,** 539–543.
9. Wang, L. F., Zhu, G. Y., Wang, P., and Newby, B. M. Z. (2005), *Biotechnol. Prog.* **21,** 1321–1328.
10. Panke, S., Wubbolts, M. G., Schmid, A., and Witholt, B. (2000), *Biotechnol. Bioeng.* **69,** 91–100.

11. Bjorkling, F., Frykman, H., Godtfredsen, S. E., and Kirk, O. (1992), *Tetrahedron* **48,** 4587–4592.
12. Bjorkling, F., Godtfredsen, S. E., and Kirk, O. (1990), *J. Chem. Soc. Chem. Commun.* **19,** 1301–1303.
13. Klaas, M. R. G. and Warwel, S. (1997), *J. Mol. Catalysis a-Chem.* **117,** 311–319.
14. Archelas, A. and Furstoss, R. (1997), *Ann. Rev. Microbiol.* **51,** 491–525.
15. Chem Systems, Inc. Report, Assessment of the likely role of biotechnology in commodity chemical production (1988), 20–97, Tarrytown, NY.
16. Soni, B. K., Kelley, R. L., and Srivastava, V. J. (1998), *Appl. Biochem. Biotechnol.* **74,** 115–123.
17. Hilker, I., Bothe, D., Pruss, J., and Warnecke, H. J. (2001), *Chem. Eng. Sci.* **56,** 427–432.
18. Klaas, M. R. and Warwel, S. (1999), *Ind. Crops Prod.* **9,** 125–132.
19. Sober, H. A. and Harte, R. A. (eds.) (1970), *Handbook of Biochemistry*, 2nd ed. The Chemical Rubber Co., Cleveland, OH: pp. E10–E21.
20. Park, J. B. and Clark, D. S. (2006), *Biotechnol. Bioeng.* **93,** 1190–1195.
21. Park, J. B. and Clark, D. S. (2006), *Biotechnol. Bioeng*. **94,** 189–192.
22. Panke, S., Held, M., Wubbolts, M. G., Witholt, B., and Schmid, A. (2002), *Biotechnol. Bioeng.* **80,** 33–41.
23. van de Velde, F., Bakker, M., van Rantwijk, F., Rai, G. P., Hager, L. P., and Sheldon, R. A. (2001), *J. Mol. Catalysis B-Enzymatic* **11,** 765–769.
24. Andersson, M., Andersson, M. M., and Adlercreutz, P. (2000), *Biocatalysis Biotransform.* **18,** 457–469.
25. Conesa, A., van de Velde, F., van Rantwijk, F., Sheldon, R. A., van den Hondel, C., and Punt, P. J. (2001), *J. Biol. Chem.* **276,** 17,635–17,640.
26. Rai, G. P., Zong, Q., and Hager, L. P. (2000), *Isr. J. Chem.* **40,** 63–70.
27. Ayala, M., Horjales, E., Pickard, M. A., and Vazquez-Duhalt, R. (2002), *Biochem. Biophys. Res. Commun.* **295,** 828–831.
28. Borole, A., Dai, S., Cheng, C. L., Rodriguez, M., and Davison, B. H. (2004), *Appl. Biochem. Biotechnol.* **113–116,** 273–285.
29. van Rantwijk, F. and Sheldon, R. A. (2000), *Curr. Opin. Biotechnol.* **11,** 554–564.