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REVIEW

Development of Enzyme Technology and Enzyme Engineering in China

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Abstract

Enzymes have been utilized in China for thousands of years for the production of various foods and alcoholic beverages. Today China manufactures and uses enzymes for not only the traditional areas of application, but is expanding the use of enzymes for a variety of nonfood areas. This report describes the present state of the art of enzyme manufacture and application in China today.

Index Entries: Enzymes; alpha-amylase; beta-amylase; glucoamylase; China; isoamylase; alpha-galactosidase; proteases; lipase; glucoepoxidase; L-asparaginase; cellulase; immobilization.

Introduction

Long before the nature of microorganisms and enzymes was made clear, the Chinese people were familiar with the utilization of the microbial activity in food processing. About three or four thousand years ago, some kinds of alcoholic beverages were made with the aid of "qu" (koji, as Japanese called it), moldy cereal containing molds and yeasts that act as starch saccharifiers and alcohol producers, respectively. Even today, the world-famous Shaoxing Shanniang (rice wine) and Maotai (spirits) are produced by such traditional fermentation processes. Our ancestors also used another kind of "qu," rich in microbial proteolytic enzymes, to hydrolyze soybean protein for the production of delicious soybean paste. Nowadays, traditional Chinese foods, such as rice wine, fermented glutinous rice, soybean paste, soybean sauce, fermented soybean, fermented soybean curd, and so on are still widely accepted among the people.

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In contrast to the past, we must confess that our modern science and technology in the field of fermentation are somewhat behind those of the developed countries. The utilization of microbial enzymes did not begin to make substantial progress until 1964, when the first factory of producing commercial enzyme products was set up. It is Wuxi Enzyme Factory and the first enzyme product was alpha-amylase. Over the last decade, there have been about twenty microbial enzyme producing factories and workships distributed in many provinces and cities. These factories produce various sorts of enzyme products, such as alpha-amylase, glucoamylase, proteinase, lipase, and glucose oxidase. These products have been applied in food processing, fermentation, leather and fur processing, and the textile, chemical, and pharmaceutical industries. The main features are summarized in Table 1.

1. Alpha-Amylase (1, 2)

Alpha-amylase was the first industrial enzyme, and still holds first place in annual output. It is produced mainly by means of submerged cultivation of *Bacillus subtilis* BF 7658, and recovered by spray-drying or ammonium sulfate precipitation. Alpha-amylase is used for textile desizing, but at present, mainly for the production of a sweet syrup that partly substitutes sucrose and improves the color and flavor of foods. In addition, alpha-amylase is also used as a starch liquefaction agent in brewing, distillery, glucose production, and some other fermentation industries where it is used as a raw material.

2. Beta-Amylase (3, 4)

A strain of *Bacillus polymyxa* producing beta-amylase has been reported. It may be used for the production of maltose or as a substitute for malt in brewing.

3. Glucoamylase

Glucoamylase, along with alpha-amylase and proteinase, are the major industrial enzymes in terms of their annual output. There are two strains of *Aspergillus niger*, M 85 (*5*), AS 3.4309 (*6, 7*), used in submerged culture, and a strain of *Monascus sp* (*8*) As 3.978 had been used. The major use of glucoamylase is to produce glucose and glucose syrup, and it is also used in brewing, distilling, and other fermentation industries as a saccharifying agent.

4. Isoamylase (Pullulanase)

A strain of *Aerobacter aerogenes* 10016 has been reported as an isoamylase producer (*9*). The enzyme may be used in brewing and maltose production to increase yields. Since the enzyme hydrolyzes pullulan but not glycogen, it is adequate to call it pullulanase (*10*).

5. Alpha-Galactosidase (11)

A strain of *Aspergillus ustus* has been used to produce alpha-galactosidase (melibiase)—for beet sugar production. It hydrolyzes raffinose to galactose and sucrose, and hence improves the sucrose yield.

TABLE 1
Industrial Microbial Enzymes and Their Application

Enzyme	Source	Reference	Application
Alpha-amylase	*Bacillus subtilis* BF 7658	*1*	Textile desizing; starch liquefaction; starch syrup; glucose; dextrin for paper coating; fermentation industry
	JD-32	*2*	
Beta-amylase	*Bacillus polymyxa*	*3, 4*	Maltose; beer
Glucoamylase	*Aspergillus niger* M 85	*5*	Glucose; starch syrup; fructose syrup; beverages; antibiotics; alcohol; amino acids
	AS 3.4309	*6, 7*	
	Monascus sp AS 3.978	*8*	
Isoamylase (pullulanase)	*Aerobacter aerogenes* 10016	*9, 10*	Beer; maltose
Alpha-galactosidase	*Aspergillus ustus*	*11*	Beet sugar
Proteinase			
Neutral	*Bacillus subtilis* AS 1.398	*12, 13*	Recovery of photographic film; antiinflammatory agent; leather unhairing and bating; degumming of silk
	Aspergillus terracola AS 3.942	*14*	
Alkaline	*Bacillus pumilus* 209	*15*	Detergent; unhairing; Degumming of silk
	289	*16*	
	Bacillus subtilis 2709	*16*	
Acidic	*Aspergillus usamii* 537	*17*	Fur processing; anti-inflammatory agent
	Aspergillus niger 3.350	*18,19*	
Lipase	*Candida lipolytica*[a] As 2.1203		Fat removal from silk, fur, leather, and gelatin; enhancing flavor of chocolate
	Eremothecium ashbyii Du-32	*20*	
Glucose oxidase	*Penicillum notatum* AS 3.3871	*21*	Removal of glucose from egg white; glucose determintation in urine and blood
Asparaginase	*E.coli* As 1.357	*22, 23*	Treatment of leukemia
Cellulase	*Trichoderma koningi* AS 3.4290	*24, 25*	Hydrolysis of furfural; industrial waste and sawdust; alcohol fermentation
	Trichoderma pseudokoningi EA_3-867, N_2-78	*26*	
	Trichoderma koningi AS 3.4001	*27*	

[a]Unpublished data.

6. *Proteinases*

At present, about ten kinds of proteinases have been produced. These may be, according to optimal reaction pH value, roughly divided into three groups: neutral, acidic, and alkaline. The main producers are bacteria (*12, 13, 15, 16*) and molds (*14, 17–19*), and most of the products are sold in the form of ammonium sulfate precipitated crude powder. Their major use is in the leather processing industry as unhairing agents. Bating and unhairing of pig skin by a chemical method had been used for years in leather processing, but these processes drain off large amounts of harmful waste waters and the operators must work under hazardous conditions. After the enzymatic unhairing technique was introduced, not only have working conditions been improved, but also the waste water has now become useful as fertilizer. Almost all kinds of proteinases, except the acidic ones, have been used as unhairing agents. The acidic proteinases are used mainly in the fur industry for bating. Proteinases are used also in the detergent, pharmaceutical, food processing, brewing, textile, and other industries.

7. *Lipase*

The ammonium sulfate-precipitated crude lipase preparation that was produced from *Candida lipolytica* has been used mainly as a defatting agent in the leather and fur industries. Lipase from *Eremothecium ashbyii (20)* had been used to enhance the flavor of butter and the removal of fat from silk.

8. *Glucose Oxidase (21)*

Glucose oxidase is produced from *Penicillium notatum* AS 3.3871; its partially purified liquid preparation has been used for glucose removal from egg white, and for making test paper for blood and urine sugar determinations.

9. *L-Asparaginase (22, 23)*

L-Asparaginase, one of the antigumor drugs, has been produced in a pharmaceutical factory with *E. coli* AS 1.357, and has been used for treating leukemia for many years. The enzyme has been purified and crystallized.

10. *Cellulases (24–27)*

Cellulases were produced from semisolid cultures of strains of *Trichoderma sp.* Cellulase had been used to hydrolyze cellulose-containing waste, such as furfural industrial waste and sawdust, and also used in the distillery industry to increase alcohol yield. But until now, it has not been used on a commercial scale.

At the beginning of the 1970s, research on immobilized enzymes and microbial cells started. Since then, some useful results have been achieved, and the techniques are being successfully employed in the chemical and pharmaceutical industries and for food processing.

I. Immobilization by Ionic Binding

1. Glucoamylase (28)

Glucoamylase from *Aspergillus niger* M 85 was immobilized on DEAE-Sephadex A-50 by ionic binding. The culture filtrate of *Asp. niger* was passed through a packed column of DEAE-Sephadex A-50 (0.1*M*, pH 6.0, phosphate buffer) or put together with the ion-exchanger in flask and shaken at 28–30°C for 2.5–5.0 h. DEAE-Sephadex A-50 is a much better adsorbent than ion-exchange resin 110, CM-cellulose, or DEAE-cellulose. Some of its basic properties are shown in Table 2. These are the results of a laboratory scale study. In addition, the K_m value of the immobilized enzyme for starch is 2.3%, while that of the free enzyme is 0.57%.

2. Aminoacylase (29)

Aminoacylase from rice koji (*Asp. oryzae*) was immobilized on DEAE-Sephadex A-50 (home-made). A water extract of koji was adsorbed on DEAE-Sephadex (60 mL to 1 g wet gel) at pH 7.0–7.5, and stirred overnight in a cold room. The properties of the immobilized enzyme are shown in Table 2. The enzyme was used to prepare optically active amino acids for reagents or pharmaceutical purposes. Besides the naturally occurring amino acids, *p*-methoxyphenylglycine and gamma-aminobutyric acid have been produced.

TABLE 2
Enzymes Immobilized by Ionic Binding

	Glucoamylase	Amino acylase	Glucose isomerase
Source	*Aspergills niger* M85	*Aspergillus oryzae*	*Streptomyces roseoruber* 336
Ion-exchanger	DEAE-Sephadex A-50	DEAE-Sephadex A-25	Porous, strong basic anion exchanger 290
Activity	1000 U/g[a]	700–800 μmol/h/g	2000 U/g (wet)[b]
Recovery, %	25	60–70	70–80
Optimal pH	4.0–4.5	7.0	7.4
Optimal temp., 55°C	55	70	80
Activator	Co^{2+}	Mg^{2+}, Co^{2+}	
Storage stability		Cold room, 3 months, no loss of activity	Air dried sample, 6 months, no loss of activity
Operational stability			70°C, 48 h, − 42%

[a] 1 Unit = μmol/min glucose formed.
[b] 1 Unit = 1 mg/h fructose formed.

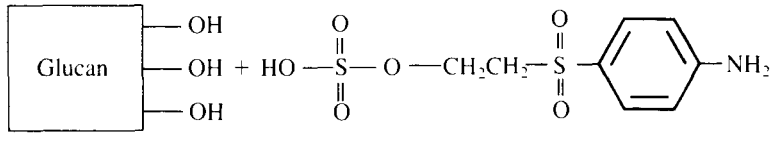
3. Glucose Isomerase

Glucose isomerase from *Streptomyces roseoruber* 336 was adsorbed on a strong basic anion exchange resin 290 (made by Nankai University). The fermentation broth had an activity of 140 U/mL. After centrifugation, the clear enzyme solution (300–400 mL) was mixed with 2.5 mL resin and 50–67 mL phospate buffer, pH 7.0 at 50°C, for 9–12 h. Some basic parameters are shown in Table 2. Pilot plant-scale experiments have been done. The productivity was about 1.25–2.2 tons of syrup per kilogram of dry material.

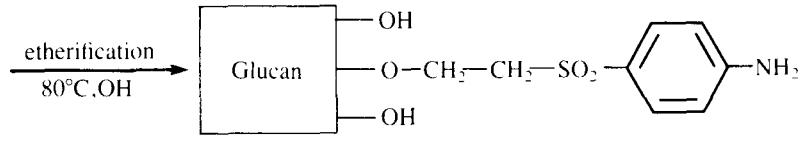
II. Covalent Binding

1. A Useful Bifunctional Reagent

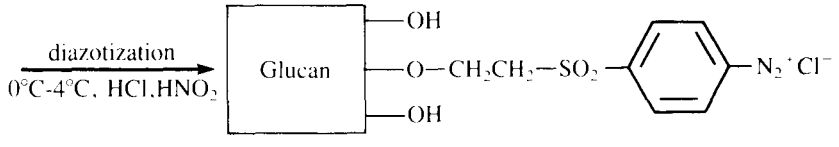
A bifunctional reagent *p*-(β-sulfatoethylsulfonyl)-aniline (SESA) is usually used in the dye industry to prepare active dyes. The commercial product is very cheap. It was first used by Prof. Tsou Chenglu (then at the Institute of Biochemistry) to prepare immobilized enzymes in 1970. Afterwards, various enzymes were bound to it with promising results. The reaction scheme is shown as follows:

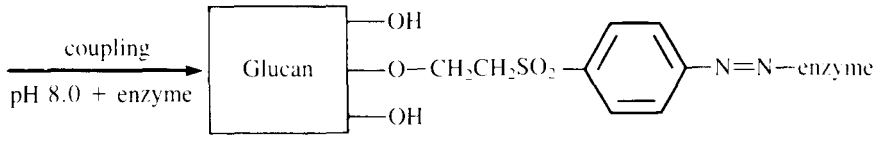


p-(β-sulfatoethylsulfonyl)-aniline(SESA)



p-aminobenzenesulfonyl ethyl glucan
(ABSE-glucan)





2. Glucoamylase Immobilized on ABSE-Cellulose (30–32)

Glucoamylase from the fermentation broth of *Asp. niger* M85 was treated with acid clay, precipitated with 75% sat. $(NH_4)_2SO_4$, and dialyzed. It was coupled to diazotized ABSE-cellulose (from bagasse). Some basic parameters are shown in Table 3. These are results of laboratory experiments.

3. Immobilized 3′-RNase (33)

Fermentation broth of *Rodotorula glutinis* was centrifuged, and $(NH_4)_2SO_4$ was added to the supernatant (600 g/L). The enzyme precipitate was dissolved in water and coupled to diazotized ABSE-Sephadex G-200. It was used for the production of 3′-mononucleotides from RNA with a tenfold increase in efficiency. Some basic parameters are shown in Table 3. The 3′-mononucleotides were used as reagents for research work and for pharmaceutical purposes.

4. Immobilized Trypsin (34)

Crystalline beef pancrease trypsin was coupled to ABSE-cellulose in the presence of *n*-butylamine (a competitive inhibitor of trypsin) to protect its active sites. It formed an inactive complex with mung bean trypsin inhibitor in a molar ratio of 2 : 1 with 80% of the active sites participating. Some properties of the immobilized enzyme are shown in Table 3. Since the carrier is cheap, industrial application may be anticipated.

5. Polynucleotide Phosphorylase (PNPase) (35, 36)

PNPase catalyzes the conversion of IDP and CDP to poly I and poly C, which combine to form poly I:C, an interferon inducer. Because it was being produced for pharmaceutical purposes, the enzyme was first purified from an *E. coli* extract by streptomycin precipitation, followed by $(NH_4)_2SO_4$ fractionation and DEAE-cellulose column chromatography. The purified enzyme was coupled to diazotized ABSE–agarose. Some properties are shown in Table 3. The immobilized PNPase has been used in a pharmaceutical factory to produce poly I:C.

6. Nuclease P_1 for the Production of 5′-Nucleotides (37)

Nuclease P_1 was obtained from the culture filtrate of *Penicillium citrinum* by alcohol precipitation. It was then coupled to ABSE-cellulose (from bagasse). Some basic properties are shown in Table 3. Pilot plant experiments carried out in 1976 were successful, and industrial production of 5′-nucleotides was achieved in 1977. It was the first industrialized immobilized enzyme in China.

7. Alkaline Phosphatase (38)

An enzyme preparation of alkaline phosphatase from *E. coli* with activity of 1200–1900 U/mL was used as the starting material. It was coupled to ABSE-agar gel beads, which had been crosslinked with epichlorohydrin, in the presence of bovine serum albumin and then treated with beta-naphthol, which was used here as a "blocking" agent. Some properties are shown in Table 3. It was satisfactorily used in the research work on nucleic acid synthesis and oligonucleotide sequencing.

TABLE 3
Enzymes Immobilized on ABSE-Glycans

	Glucoamylase	3′-RNase	Trypsin	PNPase	Nuclease P_1	Alkaline phosphatase
Source	*Aspergillus niger* M85	*Rodotorula glutinis*	Beef pancreas	*E. coli* AS 1.182	*Penicillum citrinum*	*E. coli*
Carrier	Cellulose	Sephadex G-200	Cellulose	Agarose beads	Cellulose	Agar
Activity	1000 U/g			8 U/g[b] (wet)	2090 U/g[c] (dry)	80 U/g[d]
Relative %	20–30	50	Casein: 40–50 BANA:[a] 95–100		50	
Recovery, %		35		15	19	60–80
Optimal pH	4.5	4.1		10	4.8 (5.1)[e]	8.4
Optimal temp., °C	65(70)	55		42–52	70–75 (75)[e]	68 (60)[e]
Storage stability					4°C, 1 year, no loss of activity, 37°C, 1 year, − 40%	4°C, 6 months, no loss of activity

Operational stability	After 325 h, −25%	Used for 30 times, a little decrease	More stable than free enzyme to heat and urea	No loss of activity in column reactor at pH 9, 37°C, for 1.5–2 months	Ten times stabler than the free enzyme	30°C, 40 days, no loss of activity
Application	31–34% starch glucose DE 94	RNA 3′-mono-nucleotides	For research work	Production of poly I:C from IDP and CDP	RNA 5′-mono-nucleotide yield, 42%	As a reagent for tRNA synthesis

[a]BANA: *n*-benzoyl-arginine β-naphthylamide.
[b]1 unit: $A_{257\ nm} = 1.0$.
[c]1 unit: $A_{260\ nm} = 1.0$.
[d]1 unit: $A_{410\ nm} = 0.1$.
[e]Figure in bracket is the value for free enzyme.

III. Immobilized Microbial Cells

1. Immobilized E. coli Cells with Aspartase Activity (39)

A strain of *E. coli* AS 1.881 having very high aspartase activity (1×10^5 U/g wet cells) was immobilized by entrapping the wet cells in 6% agar gel. Some properties are shown in Table 4. The efficiency is quite high, since only 80 g of wet cells can convert 1000 L of 1*M* fumarate into 110 kg L-aspartic acid in 20 days.

2. Immobilized Yeast Cells with Fumarase Activity (40)

Candida rugosa C90 have been used to produce fumaric acid from liquid paraffin. The cells harvested from the fermentation broth are able to convert fumaric acid to L-malic acid at alkaline pH. Cells were entrapped in 15% polyacrylamide gel. Some properties of the immobilized material are shown in Table 4. A reactor containing 6 g wet calls running at 30°C for 60 days can convert 12 L of 1*M* fumarate to L-malate with a yield of 82–85%. A pilot plant experiment has been completed and industrial production has been achieved.

3. Immobilized E. coli Cells with Penicillin Acylase Activity (41–43)

E. coli strain AS 1.76 has high penicillin acylase activity. The cell slurry of *E. coli* was thoroughly mixed with an equal volume of 8% agar gel and poured into an organic solvent with stirring to make gel beads. After washing, the beads were crosslinked with 1% glutaraldehyde solution and washed. Some properties of the immobilized material are shown in Table 4. Cells of *E. coli* strain D816 entrapped in gelatin gel and crosslinked with glutaraldehyde were also very useful, as shown in Table 4. Both immobilized materials have been successfully used in pharmaceutical industry to produce 6-APA from penicillin G and 7-ADCA from 7-phenylacetamido-deacetoxycephalosporanic acid.

4. Immobilized Cells Having Glucose Isomerase Activity (44)

Cells of *Streptomyces roseoflulvus* Kc 13-5705 were entrapped in gelatin and crosslinked with glutaraldehyde. Some basic parameters of the immobilized material are shown in Table 4. Pilot plant scale experiments have been completed. The efficiency of the immobilized cells was fivefold higher than that of the native cells.

5. Immobilized Microbial Cells Having 3-Ketosteroid-Δ^1-dehydrogenase Activity (45)

Cells of *Arthrobacter simplex* entrapped in 10% polyacryamide gel, or in a mixed gel composed of 5% calcium alginate and 5% gelatin (2:1), were used to convert hydrocortisone to prednisolone, or cortisone acetate to prednisone acetate. Some basic parameters are shown in Table 4. One gram of dry material gives 791.7 mg product per day.

TABLE 4
Gel-Entrapped Microbial Cells

	Aspartase	Fumarase	Penicillin	Acylase	Glucose isomerase	3-Ketosteroid-Δ^1-dehydrogenase
Source	*E. coli* AS 1.881	*Candida rugosa*	*E. coli* AS 1.76	*E. coli* D 816	*Streptomyces rosefulvus*	*Arthrobacter simplex*
Gel	6% agar	15% PA[a]	4% agar, 0.5–1.0% glutaraldehyde	Gelatin glutaraldehyde	Gelatin, 0.25% glutaraldehyde	10% PA[a]
Activity	6×10^4 U/g[b] (wet)	7000 U/g[b] (wet)				55 U/g[c] (wet)
Recovery, %	71	90			40	50
Optimal pH	9.0–9.5 (9.0)	8.5	8.0	9.0	7–8	7.0–8.5
Optimal temp., °C	40	45	40	55	85	35 (30)
Storage stability	4°C, 35 days + 180%	4–6°C, 91 days no loss of activity	4°C, 14 months, no loss of activity			−22°C, 5 months no loss of activity
Operational stability	37°C, 20 days, −18%	Half life, 95 days	Column reactor, 115 days, no loss of activity	37°C, 103 days, no loss of activity	After 20 days, conversion rate decreases to 40%	
Application	Fumarate ↓ L-aspartate	Fumarate ↓ L-malate	Production of 6-APA 7-ADCA		Glucose ↓ fructose (conversion rate, 45%)	Hydrocortisone ↓ prednisolone

[a]PA = polyacrylamide gel.
[b]1 unit: 1 μmol/h substrate change.
[c]1 unit: 1 μmol/h product formed.

IV. Micellaneous

Research work on other immobilized enzymes or microbial cells and new carriers has been undertaken. An ABSE derivative of polyvinyl alcohol (in bead form) has been used to immobilize trypsin (*46*). Isothiocyano derivatives of ABSE-cellulose have been used to immobilize alkaline phosphatase (*47*). *E. coli* having glutamate decarboxylase activity entrapped in a gel has been used to produce gamma-aminobutyric acid (*48*). Immobilized T_4 RNA ligase has been reported (*49*). Glucoamylase immobilized on porous glass will be reported soon (*50*). Other work is in progress.

References

1. Wusih Fermentation Plant for Enzymes; Institute of Chemical Engineering, Kiangsu; Wusih Light Industry College and Institute of Genetics, Academia Sinica (1976), *Yi Ch'uan Hsueh Pao* **3**(3), 216.
2. Jinan Towel Factory (1974), *Wei Sheng Wu Hsueh T'ung Pao* **1**(4), 29.
3. Guo, Jun-jun, Jiang, Zhao-yuan, and Ho, Bing-wang (1980), *Wei Sheng Wu Hsueh T'ung Pao* **7**(2), 59.
4. Ho, Bing-wang, Guo, Jun-jun, Jiang, Zhao-yuan, and Zhang, Shu-zheng (1980), *Wei Sheng Wu Hsueh Pao* **20**(4), 421.
5. Research Group on Glucoamylase (Institute of Microbiology, Academia Sinica, Beijing) (1974), *Wei Sheng Wu Hsueh Pao* **14**(1), 77.
6. Research Group on Glucoamylase (Institute of Microbiology, Academia Sinica, Beijing) (1979), *Wei Sheng Wu Hsueh T'ung Pao* **6**(5), 19.
7. Research Group on Glucoamylase (Institute of Microbiology, Academia Sinica, Beijing) (1980), *Wei Sheng Wu Hsueh T'ung Pao* **7**(4), 153.
8. Ho, Bing-wang, Guo, Jun-jun, Fang, Yi-cheng, and Chang, Shu-cheng (1973), *Wei Sheng Wu Hsueh Pao* **13**(2), 142.
9. Jiangsi Scientific Research Institute of Food and Fermentation Industry (1976), *Wei Sheng Wu Hsueh Pao* **16**(4), 282.
10. Ge, Su-guo, Yang, Shou-jun, and Zhang, Shu-zheng (1980), *Wei Sheng Wu Hsueh Pao* **20**(4), 415.
11. Liu, Bo, Peng, Wan-lin, Zhao, Shu-zhen, Wang, Chun-sheng, Gao, Su-Jie, and Tian, Xiao-guang (1979), *Wei Sheng Wu Hsueh Pao* **19**(2), 225.
12. Seed Culture Group, Shanghai Xin-xing Fermentation Plant (1979), *Wei Sheng Wu Hsueh T'ung Pao* **6**(2), 10.
13. Qiu, Xiu-bao (1979), *Wei Sheng Wu Hsueh T'ung Pao* **6**(6), 45.
14. He, Bing-wang, and Xüe, Shi-jin (1959), *Wei Sheng Wu Hsueh Tung Hsün* **1**(2), 69.
15. Honan Scientific Research Institute of Leather Industry (1975), *Wei Sheng Wu Hsueh Pao* **15**(4), 330.
16. Enzyme Research Division, Laboratory of Microbiology Institute of Forestry and Pedagogy, Shenyang (1974), *K'o Hsueh T'ung Pao* **19**(1), 34.
17. Zhou, Qin-cheng, Guan, Gui-lan, Ding, Zhi, Chen, Ju-ying, Dong, Wen-cai, Sun, Shi-zhang, and Qiu, Xiu-bau (1979), *Wei Sheng Wu Hsueh Pao* **19**(3), 315.
18. Shanghai Institute of Industrial Microbiology and The Shanghai Distillery (1976), *Wei Sheng Wu Hsueh Pao* **16**(2), 156.

19. Shanghai Institute of Industrial Microbiology and The Shanghai Distillery (1976), *Wei Sheng Wu Hsueh Pao* **16**(3), 220.
20. Shen, Yung-chiang, Liu, Tzu-chün, Chang, Ching-lu, and Wang, Li-wen (1974), *Wei Sheng Wu Huseh Pao* **14**(1), 95.
21. Cooperative Research Group of Glucose Oxidase (1974), *Wei Sheng Wu Huseh T'ung Pao* **1**(1), 1.
22. Qiu, Xiu-bau, Hao, Fong-xi, Qian, Shi-jun, Chen, Jia-yi, and Mong, Guang-zhen (1973), *Wei Sheng Wu Hsueh Pao* **13**(1), 59.
23. Mong, Guang-zhen, Qian, Shi-jun, Hao, Feng-xi, Chen, Jia-yi, and Qiu, Xiu-bau (1973), *Wei Sheng Wu Hsueh Pao* **13**(2), 102.
24. Cellulase Research Group, Institute of Microbiology, Academia Sinica and The Beijing Toiletry, Chemicals, Second Factory (1977), *Wei Sheng Wu Hsueh Pao* **17**(2), 137.
25. Cellulase Research Group, Institute of Microbiology, Academia Sinica and The Beijing Toiletry Chemicals, Second Factory (1977), *Wei Sheng Wu Hsueh Pao* **17**(3), 231.
26. Cellulase Research Laboratory, Shanghai Institute of Plant Physiology, Academia Sinica and The Shanghai Distillery No. 2 (1978), *Wei Sheng Wu Hsueh Pao* **18**(1), 27.
27. Cellulase Research Group, Institute of Microbiology, Academia Sinica (1979), *Wei Sheng Wu Huseh T'ung Pao* **6**(3), 16.
28. Research Group of Immobilized Enzyme, Institute of Microbiology, Academia Sinica (1973), *Wei Sheng Wu Huseh Pao* **13**(1), 25.
29. Yuan, Zhong-yi, et al., The Third Meeting of Chinese Biochemical Society (1978), Abstract, p. 174.
30. Li, Kao-hsiang, et al. (1973), *Wei Sheng Wu Hsueh Pao* **13**(1), 31.
31. Yang, Lian-wan, et al. (1976), *Wei Sheng Wu Hsueh Pao* **16**(4), 335.
32. Li, Gao-xiang, et al. (1979), *Wei Sheng Wu Hsueh Pao* **19**(2), 150.
33. Immobilized Enzyme Group, Shanghai Institute of Biochemistry, Academia Sinica (1977), *Sheng Wu Hua Hsueh Yu Sheng Wu Wu Li Hsueh Pao* **9**(2), 187.
34. Immobilized Enzyme Group, Shanghai Institute of Biochemistry, Academia Sinica (1977), *Sheng Wu Hua Hsueh Yu Sheng Wu Wu Li Hsueh Pao* **9**(1), 63.
35. Yang, Kai-yu, et al. (1979), *Sheng Wu Hua Hsueh Yu Sheng Wu Wu Li Hsueh Pao* **11**(1), 79.
36. Yang, Kai-yu, et al. (1979), *Sheng Wu Hua Hsueh Yu Sheng Wu Wu Li Hsueh Pao* **11**(2), 97.
37. Yuan, Zhong-yi, et al. (1980), *K'o Hsueh T'ung Pao* **25**(14), 654.
38. Yuan Zhong-yi, et al. (1982), K'o Hsueh T'ung Pao, in press.
39. Meng, Guang-zhen, et al. (1978), *Wei Sheng Wu Hsueh Pao* **18**(1), 39.
40. Yang, Lian-wan et al. (1980), *Wei Sheng Wu Hsueh Pao* **20**(3), 296.
41. Sun, Wan-ru, et al. (1980), *Wei Sheng Wu Hsueh Pao* **20**(40), 407.
42. Sun, Wan-ru, et al. (1981), *Wei Sheng Wu Hsueh T'ung Pao* **8**(2), 63.
43. Wang, Qing-cheng, et al. (1980), *Sheng Wu Hua Hsueh Yu Sheng Wu Wu Li Hsueh Pao* **12**(4), 305.
44. Immobilized Enzyme Group, Shanghai Institute of Biochemistry, Academia Sinica, et al. (1980), *Sheng Wu Hua Hsueh Yu Sheng Wu Wu Li Chin Chan* **1980**(2), 65.
45. Yang, Lian-wan, et al. (1982), *Wei Sheng Wu Hsueh Pao,* in press.
46. Li, Fu-mian et al. (1980), *K'o Hsueh T'ung Pao* **25**(15), 695.
47. Immobilized Enzyme Research Group, Institute of Microbiology (1976), *Sheng Wu Hua Hsueh Yu Sheng Wu Wu Li Hsueh Pao* **8**(3), 207.

48. Li, Yong feng (1980), *Sheng Wu Hua Hsueh Yu Sheng Wu Wu Li Chin Chan* **1980**(3), 55.
49. Tan, Shen-cao, and Hu, Mei-hao (1981), *K'o Hsueh T'ung Pao* **26**(19), 1215.
50. Li, Gao-xing, Huang, Jio-yu, Kou, Xiu-fen, and Zhang, Shu-zheng, submitted to this Journal.