

Organic Chemicals from Bioprocesses in China

Jin Huang, Lei Huang, Jianping Lin, Zhinan Xu, and Peilin Cen

Abstract Over the last 20 years, China has successfully established a modern biotechnology industry from almost nothing. Presently, China is a major producer of a vast array of products involving bioprocesses, for some China is even the world's top producer. The ever-increasing list of products includes organic acids, amino acids, antibiotics, solvents, chiral chemicals, biopesticides, and biopolymers. Herein, the research and development of bioprocesses in China will be reviewed briefly. We will concentrate on three categories of products: small molecules produced via fermentation, biopolymers produced via fermentation and small chemicals produced by enzyme-catalyzed reactions. In comparison with the traditional chemical process, in which, nonrenewable mineral resources are generally used, products in the first and second categories noted above can use renewable bioresources as raw materials. The bioprocesses are generally energy saving and environmentally benign. For products developed via the third category, although the raw materials still need to be obtained from mineral resources, the biocatalysts are more effective with higher selectivity and productivity, and the bioprocesses occur under ambient temperature and pressure, therefore, these are "green processes." Most of the products such as citric acid, xanthan and acrylamide etc., discussed in this paper have been in large-scale commercial production in China. Also introduced herein are three scientists, Prof. Shen Yinchu, Prof. Ouyang Pingkai and Prof. Chen Guoqiang, and six enterprises, Anhui Fengyuan Biochemical Co. Ltd., Shandong Hiland Biotechnology Co. Ltd., Shandong Fufeng Fermentation Co. Ltd., Shandong Bausch & Lomb-Freda Pharmaceutical Co. Ltd., Zhejiang Hangzhou Xinfu Pharmaceutical Co. Ltd., and Changzhou Changmao Biochemical Engineering Co. Ltd.; they have all contributed a great deal to research and development in the commercialization of bioprocesses.

J. Huang, L. Huang, J. Lin, Z. Xu, and P. Cen (✉)

Department of Chemical Engineering and Biological Engineering, Zhejiang University (Yuquan Campus), Hangzhou 310027, People's Republic of China
e-mail: cenpl@zju.edu.cn

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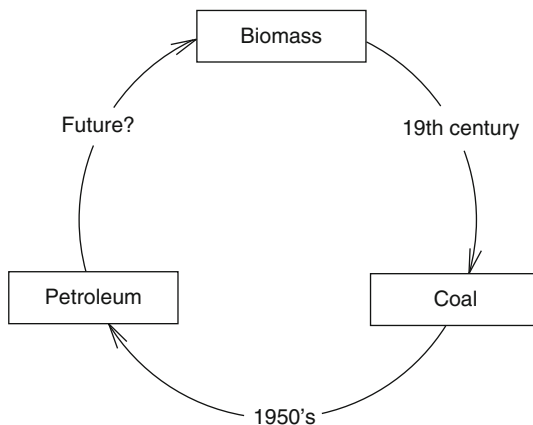
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1 Introduction

China is a vast country with a population of 1.3 billion, rich natural resources and a long history of civilization. About 5,000 years ago, the Chinese had learnt to use microorganisms to make wine, to tan animal skin and to make various kinds of fermented food. However, China lagged the pace of development in biotechnology in modern history. From the 1980s onwards, the Chinese government made a decision to open the country to the outside world and to reform China's economic structure. Biotechnology gradually caught up with world trends. Universities, research institutes, and enterprises worked together in the research and development of biotechnology and bioprocesses. Especially in the area of industrial biotechnology, various comprehensive processes have been developed, and China has been a major producer of a significant number of organic chemicals such as citric acid, xanthan, glutamic acid, penicillin, and acrylamide for example.

Organic compounds are widely applied in various areas such as agriculture, energy, materials, environmental, medicines, commodity products and food etc. It could be said that no one can live without organic chemicals. It is interesting to note that the history of the production of organic chemicals represents a cycle as shown in Fig. 1. In ancient times, only natural mixed organic chemicals from plants, animals, and microorganisms (although unrecognized as such) were utilized as food additives, beverages, dyes, and medicines etc. In the nineteenth century, organic chemists found ways to extract pure organic chemicals from natural resources or to synthesize them mainly from coal, and the typical products were methane, furfural, benzene, phenol naphthalene, and acetylene from calcium carbide, etc. In the twentieth century, petroleum, instead of coal, became the main

Fig. 1 Historic view of the production of organic chemicals



source of organic chemicals in much larger scale and over a broader product spectrum. Now, in the twenty-first century, the price of petroleum is rocketing and is no longer cheap and abundant. We therefore cannot help but to find alternative ways to produce organic chemicals. Should we go back to the biomass?

Biomass is synthesized by photosynthesis, which is renewable. In China alone, the total annual productivity of biomass is estimated at more than five billion tons. The main composition of biomass is carbohydrates and it is suitable for conversion to a variety of organic chemicals. If only 10% of the biomass is applied to the production of organic chemicals, it should be more than enough to satisfy current requirements. The conversion of biomass into organic chemicals can be carried out by gasification, high-temperature cracking or bioconversion. Bioconversion is performed at ambient temperature and pressure, the processes are energy efficient and environmentally benign. A great deal of effort has been paid to the development of new processes for the production of organic chemicals with biotechnology in China. Many review papers have been published on the research and development of industrial biotechnology in China [1–3].

Biomass-derived carbohydrates include starch, hemicelluloses, and cellulose. They are suitable to be applied as carbon sources for microorganism growth after proper pretreatment. The metabolites produced by the microorganisms can be divided into two categories: primary and secondary metabolites.

The primary metabolites are generally produced during energy metabolism. Taking glucose as an example of the carbon source, the primary metabolites are related to the EMP (Embden–Meyerhof–Parnas) pathway and tricarboxylic cycle. Various kinds of organic acids, alcohols, amino acids and nucleotides etc. belong to this category. Because of the carbohydrate nature of biomass, there are abundant oxygen atoms in the molecule of the carbon source. If the target primary metabolite is an oxygen-rich molecule, such as citric acid or lactic acid, the conversion ratio will be very high, whereas, it will be low if a hydrocarbon is predicted. The reductance degree [4] – which is defined as the number of equivalents of available electrons per gram atom carbon – is for carbon +4, hydrogen +1, oxygen –2, and nitrogen –3,

Table 1 Reductance degrees of carbon sources from biomass, and primary metabolites as well as their theoretical and practical productivities (glucose as carbon source)

	Name	Molecular formula	Reductance degree	Productivity, $Y_{p/s}$ (g/g)	
				Theoretical	Practical
Substrate	Glucose	CH_2O	+4.00		
	Xylose	CH_2O	+4.00		
	Glycerol	$\text{CH}_{8/3}\text{O}$	+4.66		
Product (glucose as substrate)	Methane	CH_4	+8.00	0.16	<0.1
	Butanol	$\text{CH}_{2.5}\text{O}_{0.25}$	+6.00	0.41	0.20
	Ethanol	$\text{CH}_3\text{O}_{0.5}$	+6.00	0.51	0.48
	Lactic acid	CH_2O	+4.00	1.0	0.90
	Citric acid	$\text{CH}_{8/6}\text{O}_{7/6}$	+3.33	1.42	1.20

and can be used as a criterion to estimate the yield of primary energy metabolites. The reductance degrees of several carbon sources from biomass, and primary metabolites as well as their theoretical and practical productivities are listed in Table 1.

The primary metabolites can be produced by either aerobic or anaerobic fermentation. The difference between aerobic and anaerobic fermentation is that the electron acceptor in aerobic growth of microbes is oxygen, whereas it is the organic substrate itself in anaerobic growth. Therefore, the reductance degree of primary metabolites in anaerobic fermentation is generally higher than that of the substrate. The higher reductance degree is desirable for a product such as alternative biofuel because of its higher heat of combustion, whereas, the product yield will be lower because more substrate must be consumed for reducing power.

The secondary metabolites, such as antibiotics, are generally produced by aerobic fermentation and not related to the cell growth, and their yields are relatively low. Herein, a brief review will be given of the organic chemicals produced from bioprocesses in China. The chemicals will be divided into three categories: small molecules, biopolymers and those produced by enzyme-catalyzed reactions.

2 Small Organic Chemicals Via Fermentation

2.1 Organic Acids from Bioprocesses in China

2.1.1 Citric Acid

In 1893, Wehmer discovered that *Penicillium* mold could produce citric acid from sugar. However, microbial production of citric acid did not become industrially important until World War I disrupted Italian citrus exports. In 1917, the American food chemist James Currie discovered that certain strains of the mold *Aspergillus niger* could be efficient citric acid producers, and Pfizer began industrial-level production using this technique 2 years later, followed by Citrique Belge in 1929, and this is still the major industrial route to citric acid used today.

In 2007, worldwide annual production of citric acid was approximately 1,700,000 Mt (Metric ton). More than 50% is used as acidulant in beverages and some 20% in other food applications. Twenty percent is used for detergent applications and 10% for other nonfood-related applications such as cosmetics, pharmaceuticals and in the chemical industry.

In China, citric acid fermentation began about 40 years ago on a small scale. Grounded corn powder or sweet potato was used as the carbon source, which is relatively cheap, however, it made the fermentation process and the citric acid separation from the broth difficult. During that period, the fermenter was about 20 m³ and was made of carbon steel, therefore the *A. niger* for citric acid fermentation must be iron ion tolerant.

Although citric acid fermentation is a mature industry, studies on strain improvement [5], product separation process [6], and process optimization [7] are still under way in China.

Currently, more than 50% of citric acid production worldwide takes place in China. The largest citric acid producer in China is Anhui Fengyuan Biochemical Co., Ltd. Each fermenter has a volume of more than 100 m³ and is made of stainless steel; in addition computer-controlled fermentation parameters are used. The main feature of the fermentation process is that a clear fermentation broth is used after low temperature liquefaction of corn starch. Because the manufacturer could be authorized to export citric acid only if the discharge of their wastewater meets the requirement, the wastewater treatment facilities including anaerobic and aerobic treatment have been operated properly in Fengyuan facility.

Anhui Fengyuan Biochemical Co. Ltd., which is a leading enterprise dealing with biological fermentation products in China, can produce 440,000 Mt of fuel ethanol, 220,000 Mt citric acid and its salts, 30,000 Mt of L-lactic acid (for epoxyethane production), and 60,000 Mt of lysine annually. The company developed “low-temperature starch liquefaction and clear broth fermentation” technology. The citric acid output is about 17% of world production.

2.1.2 Lactic Acid

Industrially, lactic acid fermentation is performed by *Lactobacillus* bacteria or *Rhizopus oryzae*. Traditionally, lactic acid is applied in the food industry as an acidifier. Today, lactic acid is used as a monomer for producing polylactic acid (PLA) which has applications as a biodegradable plastic, and is a good option for substitution of conventional plastic produced from petroleum because of the low emission of carbon dioxide and its biodegradability. An initial significant obstacle for PLA was the production cost. In 2002, Cargill started up the first of two polymer trains with a capacity of 150 million lb per year, and the price of PLA production dropped to \$1.30/lb, which is competitive with plastics from the petroleum industry.

Lactic acid has gained importance in the detergents industry during the last decade. Being a good descaler, soap-scum remover, and a registered antibacterial agent an economically beneficial as well as environmentally beneficial trend towards safer and natural ingredients has also contributed to lactic acids importance. Another promising application of lactic acid is to produce acrylic acid via catalytic dehydration, by which 65% conversion ratio can be reached [8].

There is a long history of lactic acid fermentation in China. Qian et al. [9] reviewed current research and development of lactic acid fermentation in China. Jiang et al. [10] studied L-lactic acid fermentation kinetics of *R. oryzae*. Potato starch was used as the raw material for lactic acid fermentation [11]. Cell immobilization was also studied [12]. The separation and purification of lactic acid from the fermentation broth is a difficult task. Shi [13] and Wang [14] discussed the possibility for new separation technologies for the lactic acid separation process.

The worldwide annual production of lactic acid is more than 400,000 Mt; China alone represents about one-third of this figure. The main producers of lactic acid in China are Henan Jindan Lactic Acid Co. Ltd. and Anhui Fengyuan Biochemical Co. Ltd. The capacity of lactic acid production of Henan Jindan Lactic Acid Co. Ltd. is 100,000 Mt/year, which makes them the largest lactic acid manufacturer in China and Asia. The company has developed a high substrate concentration fermentation process. Also, they applied membrane technology for the separation and purification of lactic acid to reduce energy consumption. More than ten lactic acid production projects are currently under construction with a total capability of more than 100,000 Mt/year in China.

2.1.3 Itaconic Acid and Succinic Acid

Itaconic acid is an unsaturated diprotic acid. Characteristics of the plastic and coating, which is compounded by using 1–5% itaconic acid and styrene, include light color, easy to paint, easy separation, water-fast, and antiseptis; it can be used not only in the manufacture of high-strength enhanced plastic fiberglass, but also in the coating of carpets and book covers. *Aspergillus terreus* is generally applied for itaconic acid fermentation.

In China, several groups have been working on the improvement of itaconic acid fermentation, including a fermentation kinetic study [15], solid-state fermentation [16], strain improvement [17], and product separation [18]. Qingdao Kehai Biochemistry Company produces 10,000 Mt/year of itaconic acid, which is about 50% of the total production capability in China or 18% of worldwide production.

Succinate is a component of the citric acid cycle and is capable of donating electrons to the electron transport chain. It can yield acyl halides, anhydrides, esters, amides, and nitriles for drug applications, agriculture, and food products, and other industrial uses. Succinic acid producers include *Actinobacillus succinogenes*, *Anaerobiospirillum succiniciproducens*, *Mannheimia succiniciproducens*, and recombinant *Escherichia coli*.

On January 22, 2010, DNP Green Technology and Agro-industrie Recherches et Développements of France announced that the world's first succinic acid production by biotechnology was successfully put into operation with an annual output of 2,000 Mt in their joint venture Bioamner. In China succinic acid is currently produced by chemical process, and accounts for 25–30% of the global market production volume. However, research in the field of biological processes for succinic acid production is very active. Zhang [19] and Zhan [20] reviewed recent progress in succinic acid fermentation. Sun et al. [21, 22] screened a highly productive strain, *A. succinogenes* CGMCC 1593, and the succinic acid concentration in the fermentation broth was higher than 40 g/L. Kang et al. [23] constructed an engineering cell for succinic acid production under aerobic conditions. Li et al. [24] analyzed the fermentation process for succinic acid production from crop straw hydrolysate with a neural network method. A succinic acid production project of 30,000 Mt/year is planned in Dewei, Jilin Province.

2.1.4 Long Carbon Chain Dicarboxylic Acids

The long carbon chain dicarboxylic acids ($n = 10\text{--}21$) are found in different plant lipids. It was shown that hyperthermophilic microorganisms specifically contained a large variety of dicarboxylic acids. China is the first country to commercially produce long carbon chain dicarboxylic acids by using biotechnology. In comparison with the chemical method, the biological oxidation reaction can be carried out at ambient temperature and pressure with less byproducts and lower cost. Researchers in Tsinghua University, Institute of Microbiology [25, 26], Chinese Academy of Sciences [27, 28], and the Fushun Research Institute of Petroleum and Petrochemicals [29, 30] have worked on research and development of the biological process for long carbon chain dicarboxylic acid production. After significant work in strain screening and breeding, process optimization, product separation and purification, one kg of long carbon chain dicarboxylic acid can be produced from 1.2 to 1.5 kg of alkane. Several commercial production facilities have been constructed, such as Shangdong Kaisai Biological Technology Material Co. Ltd. (16,000 Mt/year) and Shangdong Hiland Biotechnology Co. Ltd. (10,000 Mt/year).

Shandong Hiland Biotechnology Co. Ltd., has finished first-phase construction with an annual production capacity of 10,000 Mt of long carbon chain dicarboxylic acid. The biological process, initially developed by the Institute of Microbiology, Chinese Academy of Sciences, uses a strain of *Candida* sp. to oxidize light gas oil to produce dicarboxylic acid. DC11–DC18 long carbon chain dicarboxylic acids and their derivatives are important intermediates of chemical synthesis and major raw materials for synthetic Musk-T, copolyamide, melt adhesives, high-end engineering plastic, etc., and also widely applied in pesticides, pharmaceuticals, liquid crystal materials, etc.

2.2 *Alcohols from Bioprocesses*

2.2.1 Acetone/Butanol/Ethanol Fermentation

ABE fermentation (to produce acetone, butanol, and ethanol) has a long history in the world as a successful industrial fermentation process. The earliest work on this method of fermentation was performed by Pasteur in 1882, who studied the production of butanol from lactic acid and calcium lactate [1]. Commercial production attracted interest in 1909 in England because of the possibility of making synthetic rubber via butadiene production from butanol, and DuPont invented nitrocellulose layers with butyl acetate as a solvent for the automobile industry. Acetone also found application in producing explosive cordite. Weizmann successfully developed the ABE fermentation process [31–33]. During 1940, over 45×10^6 and 90×10^6 kg of acetone and butanol, respectively, were produced worldwide. However, competition from the petrochemical industry caused a decrease of the use of ABE fermentation. During the 1960s, ABE fermentation facilities were shut down worldwide except for small-scale production in China and South Africa.

The first ABE fermentation facility in China began operation in the North China Pharmaceutical Factory in 1956. The butanol was applied to the production of butyl acetate, which was used in a penicillin separation process. Another enterprise to use ABE fermentation was the Shanghai Solvent Factory which began operation in the 1950s. The annual total solvent productivities were only a few thousand tons each. With the increasing requirement of solvent in both the chemical and pharmaceutical industry and the backward petrochemical industry, ABE fermentation facilities gradually expanded in the following years. In 1995, the total number of ABE fermentation facilities in China reached more than 50 with a total annual solvent productivity of more than 100 thousand tons. The largest facility was located in the North China Pharmaceutical Factory with an annual total solvent capacity of 20,000 tons. The hydrogen produced in ABE fermentation as a byproduct was recovered and used in the production of sorbitol from glucose. The facility was operated for less than half a year because of competition from the petrochemical industry. Apparently only one factory remained in operation in 2004.

New motivation for the use of ABE fermentation began after it was found that butanol can be used as an ingredient of diesel [34] and because of the sky-rocketing price for crude oil. Recent years have seen vigorous research and development programs in ABE fermentation in China [35–38]. Now, old facilities have been reopened for production and new ones are under construction in China. Henan Tianguan Group Co. Ltd. has signed a cooperation agreement with the Shanghai Institute of Life Science, Chinese Academy of Science, to develop a new acetone/butanol/ethanol fermentation process.

2.2.2 1,3-Propanediol

1,3-Propanediol (PDD) can be formulated into a variety of industrial products including composites, adhesives, laminates, coatings, moldings, aliphatic

polyesters, and copolyesters. It is also a solvent and used as an antifreeze and wood paint.

There are two routes involving a bioprocess with certain microorganisms: (1) Conversion from glucose affected by a genetically modified strain of *E. coli*, which was developed by DuPont Tate & Lyle Bioproducts. An estimated 120,000 tons were produced in 2007. According to DuPont, the Bio-PDD process uses 40% less energy than conventional chemical processes, and reduces greenhouse gas emissions by 20%. (2) Conversion from glycerol (a byproduct of lipid processing or biodiesel production) using *Clostridium diolis* bacteria. With the development of biodiesel production, more and more glycerol will be produced. It is necessary to find a way to comprehensively utilize glycerol. The biological conversion of glycerol into 1,3-PDD is a promising solution.

In 2000 Wang et al. [39] reviewed the production processes of 1,3-PDD and indicated guidelines for research and development of 1,3-PDD. Chen et al. studied 1,3-PDD fermentation with immobilized cells [40, 41]. Scientists in Tsinghua University [42, 43] and Dalian University of Technology [44] are working hard to develop new 1,3-PDD production lines in China. A group of scientists in Tsinghua University developed a two-step fermentation process to produce 1,3-PDD from low-grade starch such as cassava. In the first step, glucose is fermented into glycerol, then the fermentation broth is inoculated with *C. diolis* to convert glycerol into 1,3-PDD. In the separation and purification process, electrodialysis technology was adopted for desalting purposes in the presence of large amounts of organic acids. The purity and recovery of 1,3-PDD reached 99.92% and 80%, respectively. Pilot-scale facilities have been successfully operated, and a 25,000 Mt/year unit is under construction. Production of 1,3-PDD from glycerol by recombinant bacteria expressing recombinant diol dehydratase was also carried out successfully.

3 Biopolymers from Bioprocesses in China

The second category of fermentation products is biopolymers. Biopolymers are polymers produced by living organisms. Cellulose and starch, proteins and peptides, and DNA and RNA are all examples of biopolymers, in which the monomeric units, respectively, are sugars, amino acids, and nucleotides. They are very important and act as genetic materials, biocatalysts, cell membranes or cell walls as well as in energy storage. Some biopolymers, such as polyamino acid (PAA) and poly-3-hydroxybutyrate can be used as plastics, replacing the need for polystyrene or polyethylene-based plastics. Some polymers, such as xanthan and hyaluronic acid, find important applications in the food industry, pharmaceuticals, cosmetics, and even in the crude oil exploitation industry. Naturally occurring biopolymers are renewable, therefore, the use of biopolymers would create a sustainable industry. In contrast, the feedstocks for polymers derived from petrochemicals will eventually run out. In addition, biopolymers have the potential to cut carbon emissions and reduce CO₂ quantities in the atmosphere, because the CO₂ released when they degrade can be reabsorbed by crops grown to replace them. This makes them close

to carbon neutral. Some biopolymers are biodegradable: they are broken down into CO₂ and water by microorganisms. In this section, only those nonstructural biopolymers produced by microorganisms will be discussed [45].

3.1 Polyamino Acids

PAAAs are of considerable commercial interest. As biodegradable polyanionic materials their applications range from slow release agents in agriculture, to detergents, surfactants, metal adsorbents, and cosmetics [46]. PAAAs offer potential for other applications, such as in diagnostics, sustained-release matrices, microencapsulation, for plasma membrane isolation and chromosomal preparations, carriers for therapeutic protein conjugates and drug delivery systems [47]. Many drug delivery studies have explored their benefits for antitumor drug conjugates. A particularly useful PAA feature is that their *in vivo* degradation rate can be modulated by structural alterations, for example, the hydrophilicity of branch residues. Glycosylated poly(L-glutamic acid) has been proposed as a biodegradable carrier for liver-specific drug delivery. Particularly important are the very low toxicities and immunogenicities PAAAs exhibit, for example, in comparison with the acute toxicity of poly(aspartic acid): *Mw* 1,500–3,000: *LD*₅₀ >2,000 mg/L (rat, oral). These factors could result in reduced drug intake requirements, and hence more convenient administration and improved patient compliance. Certain PAAAs may also offer therapeutic benefits based on their polyanionic nature.

Three kinds of poly amino acids, poly- γ -glutamic acid, poly(ϵ -L-lysine) and multi-L-arginyl-poly(L-aspartic acid) can be synthesized by bioprocesses independent of ribosomal protein biosynthesis pathways in microorganisms. These biosynthesized polymers have attracted more and more attention because of their unique properties and various applications. Only γ -PGA and poly(ϵ -L-lysine) will be discussed in this section.

3.1.1 γ -Polyglutamic Acid

γ -Polyglutamic acid (γ -PGA) is a high molecular weight (typically >1,000,000 Da), water soluble and biodegradable polymer elaborated from *Bacillus* sp. Ivanovics was the first scientist to find γ -PGA in *Bacillus anthracis* in 1937. The molecular structure of γ -PGA is shown in Fig. 2. The microorganisms, which are generally used for γ -PGA production, can be divided into two categories: glutamic acid dependent (such as *B. anthracis*, *B. licheniformis* ATCC 9945A, *B. subtilis* IFO 3335, *B. subtilis* F-2-01, *B. subtilis* CGMCC 0833), and glutamic acid independent (such as *B. subtilis* 5E, *B. subtilis* TAM-4, *B. licheniformis* A35). Glutamic acid must be added as a precursor for γ -PGA synthesis for glutamic acid-dependent strains. The reported yield of γ -PGA in the literature is in the range of 10–60 g/L in the fermentation broth [48, 49]. In this laboratory, a strain of *B. subtilis* was

Fig. 2 Molecular structure of γ -PGA

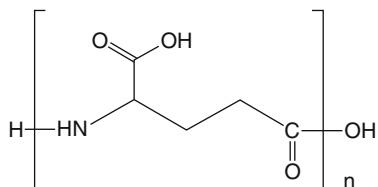
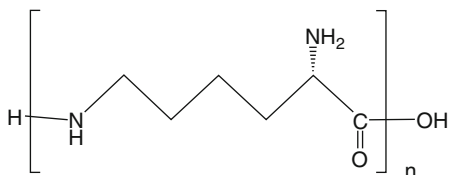


Fig. 3 Molecular structure of ϵ -PL



screened out from a Chinese traditional food: fermented bean curd. The productivity can be as high as 100 g/L and the molecular weight is about 1.3 MDa. A pilot-plant test has been successfully performed and a commercial production facility is under construction [50, 51].

3.1.2 ϵ -Polylysine

The molecular structure of ϵ -Polylysine (ϵ -PL) is shown in Fig. 3.

Production of polylysine by natural fermentation is only observed in *Streptomyces* strains. Shima et al. found ϵ -PL in the fermentation broth of *Streptomyces albulus* 346 in 1977 [52]. ϵ -Polylysine is a homo-polypeptide of approximately 25–30 L-lysine residues. The ϵ refers to the linkage of the lysine molecules. In contrast to a normal peptide bond that is linked by the α -carbon group, the lysine amino acids are molecularly linked by the ϵ amino group and the carboxyl group.

ϵ -Polylysine belongs to the group of cationic surfactants. In water, ϵ -polylysine contains a positively charged hydrophilic amino group and a hydrophobic methylene group. Cationic surface-active compounds have the ability to inhibit the growth of microorganisms. According to research, ϵ -polylysine is absorbed electrostatically to the cell surface of the bacteria, followed by a stripping of the outer membrane. This eventually leads to an abnormal distribution of the cytoplasm causing damage to the bacterial cell [53].

Because ϵ -PL can be adsorbed on the surface of microorganisms by the static electricity interaction, a powerful inhibitory effect on microbes is observed. For various kinds of microbes, the minimum inhibitory concentration (MIC) of ϵ -PL is as low as 100 μ g/mL. The toxicological study showed that ϵ -PL is safe for human beings, and even at high concentration, no toxic effect or gene mutation is observed. Therefore, ϵ -PL has been widely applied as a food preservative. Another application of ϵ -PL is as a medicine carrier. Its cationic property can enhance the ability to penetrate cell membranes [54].

There has been active research and development of the production and application of ϵ -PL in China [55, 56]. Jia et al. [57, 58] studied the effects of stirring rate and pH value on ϵ -PL formation. And after mutation and screening, the ϵ -PL concentration in a fermentation broth of *S. albulus* 410 can reach 48.3 g/L with careful pH and glucose concentration control. Zhejiang Silver-Elephant Bioengineering Co. Ltd., Zhengzhou Bainafo Bioengineering Co. Ltd., and Chengdu Jinkai biology Engineering Co. Ltd. are currently the major ϵ -PL producers in China.

3.2 Microbial Polysaccharides

Studies on microbial polysaccharides were initiated during World War II, due to the discovery of dextran, which is able to reduce blood viscosity. After this, various kinds of polysaccharides and their important functions were identified. Currently, the output of microbial polysaccharides worldwide increases more than 10% annually.

Microbial polysaccharides can be divided into three types according to their location and function in the cells [59]: (1) Intracellular polysaccharides, which store carbon and energy resources for cell growth and metabolism. (2) Structural polysaccharides to form complete cell membranes and cell walls. (3) Extracellular polysaccharides to protect cells. In this section, only three kinds of important extracellular polysaccharides, namely: Xanthan, Gellan gum (Gellan), and Hyaluronic acid (HA) will be reviewed.

3.2.1 Xanthan

The molecular structure of xanthan is shown in Fig. 4. It is composed of three kinds of monomers, namely, D-glucose, D-mannose, and D-glucuronic acid with different pyruvyl or acetyl groups and with changing ratio. The average percent composition of xanthan produced by *Xanthomonas* bacteria, which is a plant-pathogenic type of bacteria, was listed in Table 2. Synthesis originates from glucose as the substrate for synthesis of the sugar nucleotide precursors UDP-glucose, UDP-glucuronate, and GDP-mannose that are required for building the pentasaccharide repeat unit. This links the synthesis of xanthan to central carbohydrate metabolism. The repeat units are built up at undecaprenylphosphate lipid carriers that are anchored in the cytoplasmic membrane. Specific glycosyltransferases sequentially transfer the sugar moieties of the nucleotide sugar xanthan precursors to the lipid carriers. Acetyl and pyruvyl residues are added as noncarbohydrate decorations. Mature repeat units are polymerized and exported in a way resembling the Wzy-dependent polysaccharide synthesis mechanism of *Enterobacteriaceae*. Products of the *gum* gene cluster drive synthesis, polymerization, and export of the repeat unit. Average molecular weight of xanthan is in the range of 2×10^6 – 20×10^6 Da [60].

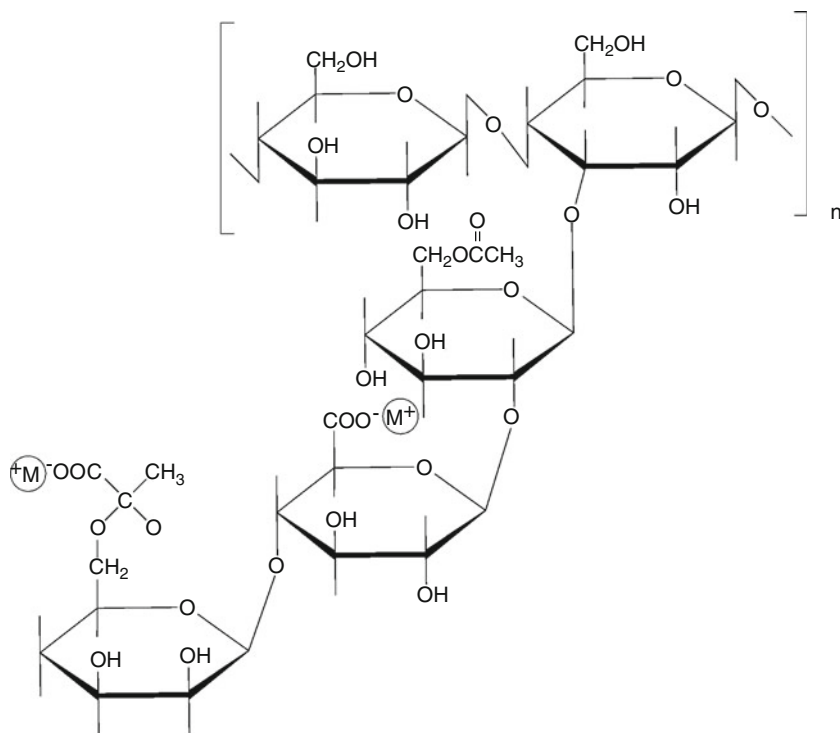


Fig. 4 Molecular structure of Xanthan

Table 2 Average percent compositions of xanthan produced by *Xanthomonas* bacteria

Bacteria	D-Glucose	D-Mannose	D-Glucuronic acid	Pyruvate	Acetate
<i>X. campestris</i>	30.1	27.3	14.9	7.1	6.5
<i>X. fragaria</i> 1822	24.6	26.1	14.0	4.9	5.5
<i>X. gummisudans</i> 2182	34.8	30.7	16.5	4.7	10.0
<i>X. juglandis</i> 411	33.2	30.2	16.8	6.9	6.4
<i>X. phaseoli</i> 1128	30.9	28.6	15.3	1.8	6.4
<i>X. vascularum</i> 702	34.9	30.2	17.9	6.6	6.3

Xanthan was discovered by an extensive research effort by a group of scientists at the United States Department of Agriculture, and was brought into commercial production by the Kelco Company under the trade name Kelzan in the early 1960s [61]. It was approved for use in foods in 1968 and has been accepted as a safe food additive in many countries. One of the most remarkable properties of xanthan gum is its ability to produce a large increase in the viscosity of a liquid by adding a very small quantity of gum, on the order of one percent. In most foods, it is used at 0.5%, or even lower. The viscosity of xanthan gum solutions decreases with higher shear rates; this is called pseudoplasticity. Unlike other gums, it is very stable under a wide range of temperatures and pH because of its branched structure with a

cellulose-like backbone. In foods, xanthan gum is most often found in salad dressings and sauces. It helps to prevent oil separation by stabilizing the emulsion, and suspend solid particles, such as spices. Toothpaste often contains xanthan gum, where it serves as a binder to keep the product uniform. In the oil industry, xanthan gum is used in large quantities, usually to thicken drilling mud. In cosmetics, xanthan gum is used to prepare water gels. Xanthan gum is a common ingredient in fake blood recipes, and in gunge.

Shandong Fufeng Fermentation Co., Ltd is a company group with more than 60 years experience in producing various bio-fermentation products. The company has been engaging in the R&D, production and marketing of fermentation products, including glutamic acid (180,000 Mt/year), xanthan gum (50,000 Mt/year), monosodium glutamate (75,000 Mt), compound fertilizer (300,000 Mt/year) etc. Having the advantages of specific experience, technology, raw material and energy sources, the company has become the largest fermentation industrial base for producing glutamic acid and xanthan in China.

The research and development of xanthan fermentation in China began 20 years ago [62]. Because of its high viscosity nature, it is necessary to overcome difficulties caused by the high viscosity such as oxygen mass transfer, stirring, and fermenter design [63, 64]. After laborious research and development, Chinese scientists and manufacturers screened highly productive strains for xanthan gum production, such as Shanda-152 and L4, enhanced oxygen mass transfer in high viscosity fermentation, and modified xanthan gum separation and purification processes. Now, xanthan production has been successfully commercialized.

Although CP Kelco currently supplies 40% of the global market for xanthan gum, China is already the largest exporter. CP Kelco and Cargill Inc., world famous xanthan gum producers, have set up xanthan gum production facilities or formed joint ventures in China. Shandong province, China, has been the base for xanthan gum production. The annual output of xanthan gum from Shandong Deosen Corporation Ltd. and Shangdong Fufeng fermentation Co. Ltd., is over 40,000 Mt each. The progress of xanthan production in China is listed in Table 3.

Table 3 Progress of annual xanthan production in China

Year	Output (Mt/year)
1992	100
1998	1,000
2003	20,000
2005	40,000
2009	>100,000

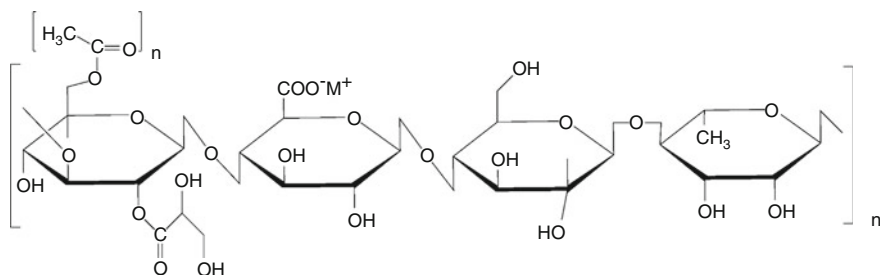


Fig. 5 Schematic diagram of Gellan molecular structure

3.2.2 Gellan Gum

The repeating unit of the gellan gum is a tetrasaccharide which consists of two residues of D-glucose and one of each residue of L-rhamnose and D-glucuronic acid [65]. The molecular structure of gellan gum is shown in Fig. 5. Compared with other colloids, gellan gum has many peculiar advantages such as: low dosage, excellent thermal and acid stability, good taste-releasing ability, high transparency, adjustable gel elasticity and rigidity and good combinability. Gellan gum, also known commercially as Phytigel or Gelrite, is used primarily as a gelling agent, an alternative to agar, in microbiological culture. It is able to withstand 120°C heat, making it especially useful in culturing thermophilic organisms. As a food additive, gellan gum is used as a thickener, emulsifier, and stabilizer. It is also used in soya milk to keep the soy protein suspended in the milk.

Gellan gum is a bacterial exopolysaccharide, prepared commercially by aerobic submerged fermentation from *Sphingomonas elodea* (previously called *Pseudomonas elodea*). It was found that inorganic nitrogen sources were favorable for cell growth and gellan production. The addition of ADP (1 mM) and tryptophan (0.05%) to the medium led to an increase in the yield of gellan up to 39.5 g/L [66].

The production and application of gellan gum in China has been reviewed [67, 68] and the fermentation process was optimized [69]. Commercial production of gellan gum has been performed in several enterprises on a small scale. The main producers of gellan gum in China are Zhejiang Zhongken Biotechnology Co. Ltd. and Shandong Anke Bioengineering Co. Ltd., with an annual output of a few hundred tons each.

3.2.3 Hyaluronic Acid

Hyaluronan (also called hyaluronic acid or hyaluronate) (HA) is an anionic, non-sulfated glycosaminoglycan distributed widely throughout connective, epithelial, and neural tissues. Hyaluronan is a polymer of disaccharides, composed of D-glucuronic acid and D-N-acetylglucosamine, linked together via alternating β -1,4 and β -1,3 glycosidic bonds. Its molecular structure is shown in Fig. 6. Hyaluronan

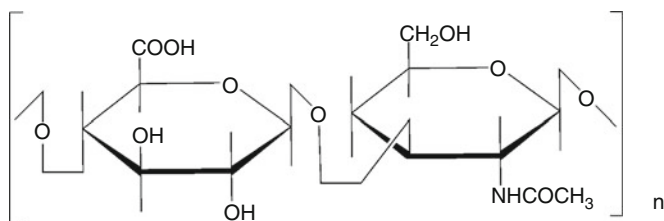


Fig. 6 HA molecular structure

can be 25,000 disaccharide repeats in length. Polymers of hyaluronan can range in size from 5,000 to 20,000,000 Da *in vivo*. The average molecular weight in human synovial fluid is three to four million Daltons. The first hyaluronan biomedical product, Healon, was developed in the 1970s by Pharmacia, and is approved for use in eye surgery. Hyaluronan is also used to treat osteoarthritis of the knee. Because of its high biocompatibility and its common presence in the extracellular matrix of tissues, hyaluronan is gaining popularity as a biomaterial scaffold in tissue engineering research. Hyaluronan is also a common ingredient in skin care products.

In 1937, Kendall et al. found that *Streptococcus haemolyticus* can biosynthesize HA [70]. Currently, HA is produced commercially by either extraction from animal tissue (i.e. rooster comb) or bacterial fermentation [71]. Increased concerns over the contamination of animal-derived products with infectious agents have made bacterial fermentation a more desirable production system to meet future demands. The high viscosity of HA dictates low titres of 5–10 g/L, a level readily achieved through batch fermentation of *Streptococci*. Strain and process development has focused on improving quality, in particular molecular weight. Little is known about what controls the molecular weight of β -polysaccharides such as HA. The HA synthase is responsible for all steps in polymerization and most likely also translocation. *In vitro* studies have identified several residues essential for high molecular weight and maximum molecular weight appears to be an intrinsic feature of the synthase. The actual molecular weight realized in fermentation, however, depends on fermentation conditions. In general, high molecular weight is observed under conditions with excess resources. Metabolic engineering and the recent advance in *omics* technologies are providing new opportunities. Heterologous hosts such as *B. subtilis*, *L. lactis*, and *E. coli* have been successfully engineered to produce HA and may prove more amenable to engineering high molecular weight HA [72].

In order to increase HA productivity, various strain improvement strategies have been adopted such as mutation by physical or chemical methods, plasma fusion and genetic engineering cell construction. After optimization of medium composition and fermentation conditions, the HA productivity increases 4.5 times compared to the original strain [73, 74]. Commercial production is successful in China. The largest manufacturers are Shandong C.P. Freda Pharmaceutical Co. Ltd. and QuFu GuangLong Biochemical Ltd.

Shandong Bausch & Lomb-Freda Pharmaceutical Co Ltd., is a joint venture of C.P. Pharmaceutical Group of Thailand, Freda International Inc. of America and Shandong Biochemical Pharmaceutical Co. of China. The company has been the leading manufacturer in the development and application of sodium hyaluronate series products. Bausch & Lomb spent \$ 200 million in cash to acquire 55% of the pharmaceutical unit in 2008.

3.3 Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHAs) are organic polyesters composed of (*R*)-3-hydroxy fatty acids which are synthesized by most bacteria as a carbon and energy storage material in times of unbalanced nutrient availability [75]. They are deposited intracellularly as insoluble spherical inclusions called PHA granules which consist of a polyester core surrounded by a phospholipid layer with attached proteins. One of these proteins is the PHA synthase, the key enzyme of PHA biosynthesis, which catalyzes polyester formation from different (*R*)-3-hydroxyacyl-CoA precursors. More than 150 different monomers can be combined within this family to give materials with extremely different properties. These plastics are biodegradable and are used in the production of bioplastics. The general chemical structure of PHAs is shown in Fig. 7.

To produce PHA, a culture of a microorganism such as *Alcaligenes eutrophus* is placed in a suitable medium and fed appropriate nutrients so that it multiplies rapidly. Once the population has reached a substantial level, the nutrient composition is changed to force the microorganism to synthesize PHA. Harvested amounts of PHA from the organism can be higher than 80% of the organism's dry weight. The biosynthesis of PHA is usually caused by certain deficiency conditions (e.g. lack of macro elements such as phosphorus, nitrogen, trace elements, or lack of oxygen) and the excess supply of carbon sources [76].

Polyesters are deposited in the form of highly refractive granules in the cells. Depending upon the microorganism and the cultivation conditions, homo- or copolyesters with different hydroxyalkanoic acids are generated.

PHA synthases are the key enzymes of PHA biosynthesis. They use the coenzyme A thioester of (*r*)-hydroxy fatty acids as the substrate. The two classes of PHA synthases differ in the specific use of hydroxyl fatty acids of short or medium chain length. The resulting PHA is of two types: (1) Poly (HA SCL) from hydroxy fatty acids with short chain lengths including three to five carbon atoms are synthesized

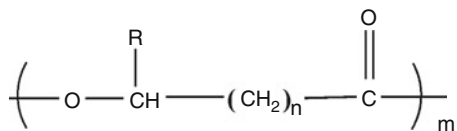


Fig. 7 The general chemical structure of PHAs

$n = 1, 2, 3, 4$; $m = 200-12000$; $R = \text{H}, \text{CH}_3, \text{C}_2\text{H}_5, \text{C}_3\text{H}_7, \dots$

by numerous bacteria, including *Ralstonia eutropha* and *Alcaligenes latus* (PHB). (2) Poly (HA MCL) from hydroxy fatty acids with middle chain lengths including 6 to 14 carbon atoms, can be made, for example, by *Pseudomonas putida*.

A few bacteria, including *Aeromonas hydrophila* and *Thiococcus pfennigii*, synthesize copolyester, from the above two types of hydroxy fatty acids or at least possess enzymes. Another even large scale synthesis can be done with the help of soil organisms. When lacking nitrogen and phosphorus they produce a kilogram of PHA from three kilograms of sugar.

The simplest and most commonly occurring form of PHA is the fermentative production of polyhydroxybutyric acid, PHB. This consists of 1,000 to 30,000 hydroxy fatty acid units. After completion of the biosynthesis, the bacteria can be up to 80% polyester by weight.

In the industrial production of PHA, the polyester is extracted and purified from the bacteria by optimizing the conditions of microbial fermentation of sugar or glucose.

The British chemical company Imperial Chemical Industries (ICI), developed in the 1980s fermentatively created copolyester produced from 3-hydroxybutyrate and 3-hydroxyvalerianacid. It was sold under the name "Biopol."

Research and development of PHA production in China can be traced back 20 years [77–80]. In the beginning, research interests were focused on strain screening and breeding as well as optimization of fermentation conditions to increase intracellular PHA yield [81]. In order to increase the productivity, high cell density cultivation was considered, however, oxygen mass transfer was found to be a limiting factor for high cell density cultivation. A group of scientists in Tsinghua University proposed a solution strategy. They constructed genetic engineering *E. coli* cells [82–84], in which, a PHB synthase gene (*phbCAB*), a *Vitreoscilla* hemoglobin gene (*vgb*) and a phage λ lysis gene (*SRRz*) were inserted into the chromosome of *E. coli*. By this method, during cell cultivation, hemoglobin was expressed to enhance the oxygen mass transfer to facilitate high cell density cultivation up to 200 gDW/L, PHB was synthesized in high yield (>90% of dried cell weight) and lyase was expressed at the end of fermentation, which was able to lyse the cell wall to facilitate the release of PHB. In this way, the production cost of PHB was reduced greatly. Several companies in China specialize in the production of various kinds of PHAs. For example, Ecomann Biotechnology Co. Ltd. set up a 5,000 MT/year facility in Shandong Province which was put into successful operation on July 29, 2009. Tianjin Green BioScience Ltd. (TGBS) is a biotech company dedicated to developing bioplastics and relevant products. By the 2008 Olympic Games, TGBS was capable of producing 10,000 tons annually.

Prof. Guo-Qiang Chen (Department of Biology Science and Technology, Tsinghua University) has focused his research on biopolyester polyhydroxyalkanoates (PHA) since 1986. He has been actively promoting the PHA-based bio- and material industries in China, including the use of bioplastics in the 2008 Beijing Olympic Games which were designated the Green Olympics,
(continued)

and the use of PHA-related products for applications such as biofuels and bulk chemicals. Prof. Chen has more than 20 years of R&D experience in PHA production and applications, and has published over 150 international peer-reviewed papers.

4 Products from Enzyme-Catalyzed Reactions in China

The third category of organic compounds produced by bioprocesses is those produced by enzyme-catalyzed reactions, which are traditionally produced by chemical-catalyzed reaction at high cost, with high energy consumption, low yield and severe environmental pollution. With enzyme-catalyzed reactions, the process can be operated at ambient temperature and pressure, with much higher efficiency and selectivity, and therefore it is a so-called “green process.” A present trend is to find specific enzymes and design specific processes to replace the traditional chemical counterpart.

In this section, several successful industrial processes using the enzyme-catalyzed reaction in China will be introduced.

4.1 Acrylamide

Shen Yinchu, a member of the Chinese Academy of Engineering, is a well-known specialist in the field of biochemical technology. He graduated from the Department of Biochemistry of Fu Dan University in 1962. Since then, he has been working in the Shanghai Pesticide Research Institute, Shanghai Research Center of Bio-chemical Technology of the Ministry of Chemical Industry and Zhejiang University of Industry. He has long been engaged in the research and development of biochemical engineering and biopesticides, and has made important contributions. During the 1980s, Shen Yinchu and coworkers carried out difficult and creative research to produce acrylamide via the enzyme-catalyzed reaction instead of the traditional chemical method. Currently all acrylamide production units in China use their technology.

Acrylamide is a monomer of polyacrylamide, which is used in synthetic fibers, oil recovery and flocculating agents, paper manufacture, and textile sizers. The conventional process for acrylamide production is based on the hydrolysis of acrylonitrile using copper-based catalysts, which requires laborious preparation, difficult purification and isolation of reaction products, and high temperature conditions [85]. Several groups of bacteria such as *Nocardia*, *Brevibacterium*, *Arthrobacter*, *Rhodococcus*, and *Pseudomonas* are able to convert acrylonitrile to acrylamide. These bacteria are known to possess a diverse spectrum of nitrile

hydratase (NHase) activities. The NHase is the biocatalyst which has been used in industrial bioconversion of acrylonitrile to acrylamide.

In 1986, Shen and coworkers from the Shanghai Pesticide Research Institute screened a high-yielding NHase strain with an orange-red appearance, which was identified as *Nocardia* sp. and tentatively named *Nocardia* sp. 86-163. The NHase activity of the strain was significantly improved up to 5627.5 U/mL through strain improvement by mutagenesis and optimization of culture conditions. Also, they developed a comprehensive process to perform the enzyme-catalyzed reaction and product separation and purification [86]. In China, the first pilot-scale unit capable of producing 440 Mt/year of acrylamide began successful operation in 1993. The first generation of commercial facilities was constructed by the Hebei Wanquan Oilfield Chemical Company and Shandong Shengli Oilfield Group with a capacity of 1,000–2,000 Mt/year acrylamide. Presently acrylamide production using chemical processes is no longer carried out; instead, the annual output of more than 200,000 MT of acrylamide is solely produced by enzymatic processes in China.

Research on strain improvement is still under way [87, 88]. Various microorganisms such as *Micrococcus* sp., *Nocardia* sp., and *Rhodococcus* sp., were selected to screen for a high NHase activity strain. It was found that with a *Nocardia* sp., the RS strain, NHase activity was increased to 10,195 U/mL in the glucose–Co²⁺ coupling fed fermentation, which is the highest among all those reported in NHase production [89].

4.2 D-Panthenol and D-Pantolactone

Biocatalytic resolutions make use of the selectivity of enzymes for one of the enantiomers of a chiral molecule, whereby one enantiomer of a racemate remains virtually untouched and the other enantiomer is converted into the desired enantiomerically pure product/intermediate. Hydrolases are by far the most prominent group of enzymes used in production of fine chemicals by biocatalytic resolution.

D-Pantolactone is a key compound in the synthesis of D-calcium pantothenate and D-panthenol [90]. Both compounds are widely used as ingredients in pharmaceutical and cosmetic compositions, as well as in food and feed supplements. Panthenol is the alcohol analog of pantothenic acid (vitamin B5), which is an “antistress vitamin” and is part of the water-soluble B-vitamin group. In organisms it is quickly oxidized to pantothenate. Panthenol comes in two enantiomers, D and L. Only D-panthenol (*dexpanthenol*) is biologically active. In cosmetics, panthenol is a humectant, emollient, and moisturizer. It is the key precursor for coenzyme A (CoA) and acyl carrier protein. Biosynthesis of pantothenate takes place only in bacteria, fungi, and plants, while animals must obtain it from their diet. Pantothenate deficiency in humans can result in abdominal distress, vomiting, cramps, burning feet syndrome, fatigue, insomnia, and reduced immunity to

some infectious agents. In 2002, the global production of pantothenates exceeded 9,000 Mt.

In the 1990s the method of production still depended for the most part on chemical synthesis from bulk chemicals. The chemical synthesis process involves reactions yielding racemic pantolactone from isobutyraldehyde, formaldehyde, and cyanide, followed by optical resolution of the racemic pantolactone to D-pantolactone, and condensation of the D-pantolactone with β -alanine. However, this synthesis requires the optical resolution of racemic intermediates. In the resolution step, the use of an expensive alkaloid as a resolving agent is unavoidable. Therefore, a variety of routes have been assayed to improve its synthesis, including stereospecific reduction of ketopantolactone [91], ketopantoate [92] or 2'-ketopantothenate derivative [93], stereoselective inversion of L-pantolactone in a racemic mixture to D-pantolactone [94] and stereoselective hydrolysis of esterified pantolactone [95]. In 1995 one of the processes of D-pantothenate synthesis used the lactonohydrolase activity of *Fusarium oxysporum*, which catalyzes the stereospecific hydrolysis of chemically made D,L-pantolactone to generate D-pantolactone as a chiral building block for its further chemical conversion to D-pantothenate [96]. The reaction is stereospecific and only the D-enantiomer in the racemic mixture is hydrolyzed. *F. oxysporum* AKU 3702 showed high productivity of the enzyme and the whole cells containing the enzyme could be used repeatedly for this hydrolysis reaction. At 30°C and pH 6.8–7.2, 90–95% of the D-pantolactone was hydrolyzed after 21 h reaction with 90–97% optical purity. The immobilized mycelia retained more than 90% of their initial activity after 180 repeated reactions [97].

Calcium pantothenate used in the feed industry in China was dependent for a long time on imports. The Shanghai Fourth Pharmaceutical Factory was the first company to produce D-calcium pantothenate as a vitamin raw material in 1958. Until 1996, however, the production capacity of China's calcium pantothenate was less than 100 MT annually. In order to meet the need of feed additives, in the 1990s, China began to develop calcium pantothenate industries and built a number of 100-ton-scale D-calcium pantothenate production plants with two unique characteristics: the use of D-amine hydrochloride, which was the byproduct of chloramphenicol production, in splitting pantolactone; and the induced crystallization method to split D,L-calcium pantothenate.

Sun et al. [98] isolated and identified a strain of *Fusarium moniliforme* SW-902 for producing D-lactonohydrolase in 2001. Using glycerol as the carbon source and peptone as the nitrogen source, in a 60 L fermentor under optimum conditions, about 7.18 g dry cell/L and 0.92 IU/g dry cell weight were obtained [99]. This technology has been applied to commercial production of D-calcium pantothenate and D-Panthenol in Zhejiang Hangzhou Xinfu Pharmaceutical Co. Ltd., China [100]. In comparison with the traditional chemical synthesis process, the biological method reduces raw material and energy consumptions by 69.2% and 12.7%, respectively. This means a higher atomic utilization ratio, low emissions, and higher profits. Now, Xinfu Pharmaceutical Co., Ltd. has become the largest producer of both Calcium D-Pantothenate and Panthenol in the world.

Zhejiang Hangzhou Xinfu Pharmaceutical Co. Ltd. is a globally leading manufacturer and supplier of Vitamin B5 (Calcium D-Pantothenate and Panthenol series products). The D-calcium pantothenate production capacity is 5,000 Mt/year, which is about 40% of the world market. Xinfu is also the only manufacturer of D-panthenol (Pro-Vitamin B5) in China.

4.3 Malic Acid and L-Tartaric Acid

Malic acid is a four-carbon dicarboxylic acid, and an intermediate of the tricarboxylic acid (TCA) cycle. Industrially, malic acid has been employed for the preparation of food additives and synthesis of various fine chemicals [101]. Malic acid is also applied in the pharmaceutical and cosmetic industries. Malic acid can be produced by various methods such as isolating it from natural fruit juices, enzymatic conversion and chemical synthesis. Commercially it is produced either by chemical synthesis via hydration of fumaric acid which results in a racemic mixture or by an enzymatic process which yields optical pure malic acid. L-malic acid is produced from fumarate by enantioselective hydration catalyzed by fumarase, using either whole cells or isolated enzyme [102, 103]. This process is a typical equilibrium reaction [104]. Malic acid can also be produced by direct fermentation with a wide range of microorganisms. Fermentation of *Aspergillus flavus* allows successful production of malic acid from renewable feedstocks, but the productivity of malic acid is low.

Brevibacterium ammoniagenses has high fumarase activity, and is suitable for malic acid production, either in free or immobilized whole cell systems. The disadvantage of this system was that byproducts like succinic acid were formed in considerable amounts. In the 1990s production of L-malic acid from fumaric acid using *Saccharomyces cerevisiae* cells was extensively studied. *S. cerevisiae* entrapped into polyacrylamide gel disks can produce malic acid from fumaric acid without formation of a byproduct. Malic acid concentration of 12 g/L was achieved in *S. cerevisiae* by overexpression of the cytosolic isoenzyme of malate dehydrogenase (Mdh2p) [105].

Ouyang et al. [106] found a strain of *S. cerevisiae*, which was high in malate hydratase activity. A new process was developed based on the principle of the coupling reaction and separation process. They [107] studied the dissolution behaviors of the substrate calcium fumarate and product calcium malate. The kinetic equations for different temperatures were proposed and the effect of pH and temperature on the dissolution process was studied. On the basis of the above work, the free cells were used as the catalyst. The slightly soluble calcium fumarate was used as the substrate. The formation of malate was able to increase the solubility of calcium fumarate, which was favorable for the enzyme-catalyzed reaction. The produced excess calcium malate formed solid crystals again and was easily removed from the reaction system. By this process, the original reversible reaction becomes favorable to malate formation, and an almost 100% conversion ratio was

achieved. In 2004 a similar process in which D-malate was effectively produced from maleate by maleate hydratase of *Pimelobacter simplex* DM18 was developed by Ouyang et al. [108]. The conversion of a high concentration of Ca-maleate into Ca-malate was achieved by maleate hydratase, owing to the low solubility of both the Ca-maleate and Ca-malate complex. The coupling reaction and separation was beneficial to both product formation and downstream processing. After 36 h of reaction, 385 g/L of Ca-D-malate was produced with an optical purity and molar yield of 97.03% and 99%, respectively.

Prof. Ouyang Pingkai, a member of the Chinese Academy of Engineering, is a leading scientist in biochemical engineering. He originally presented the combinational method to construct and optimize bioprocesses, such as the combination of bioreactions, combination of bioreaction and biomembrane, combination of bioreaction and separation. These concepts have been applied in various bioprocesses for the production of FDP, L-Alanine, L-Phenylalanine, and L-malic acid etc. in China and the highest production levels in the world were achieved.

L-tartaric acid is also a four-carbon dicarboxylic acid and a well-known natural acid that is widely distributed in many kinds of fruits, especially grapes. A broad application spectrum has been found in food, pharmaceutical and cement industries. In addition, tartaric acid also usually serves as a starting substance for numerous chemical reactions, especially for chiral synthesis. Traditionally, L-tartaric acid is produced from crude potassium tartrate which is obtained during wine making. Currently, L-tartaric acid is produced biotechnologically from *cis*-epoxysuccinic acid [109].

Various microorganisms, such as *Gluconobacter suboxydans* [110, 111], *Corynebacterium* sp. [112], *Nocardia tartaricans* [113] and *Rhodococcus rubber* [114], have been used to produce *cis*-epoxysuccinate hydrolase (CES hydrolase), which is further applied for L-tartaric acid production from *cis*-epoxysuccinic acid. The activity of CES hydrolase of the abovementioned microorganisms is stimulated by the presence of *cis*-epoxysuccinic acid in the culture medium. Whole-cell immobilization has been investigated as a method to reuse the biocatalyst and increase the productivity of the CES biotransformation process.

In 1995, Sun et al. reported that immobilized *N. tartaricans* SW 13-57 cells showed high CES hydrolase activity [113]. The optimum pH of immobilized cells was pH 8.0–9.0. With a fixed-bed column packed with immobilized cells, the average molar conversion ratio reached 99.09% after being continuously operated for 53 days. In 2000, Zhang et al. [112] immobilized *Corynebacterium* sp. JZ-1 cells by entrapping in k-carrageenan gel beads. Satisfactory results were observed in repeated batch operation and continuous fixed-bed operation for 90 days. In 2004, Min et al. [114] isolated one strain of *R. rubber* with high CES hydrolase production ability.

Changzhou Changmao Biochemical Engineering Co. Ltd. The company specializes in manufacturing C-4 series organic acids and chiral products including fumaric acid, maleic acid, L-malic acid, D-malic acid, DL-malic acid, L(+)-tartaric acid, D(-)-tartaric acid, DL-tartaric acid, L-aspartic acid, and aspartame. The total annual output reaches 25,000 Mt. In D- and L-malic acid production, the company closely cooperated with Prof. Ouyang Pingkai and Prof. Sun Zhihao, and made great progress.

5 Future Perspectives

China is among the countries that have emerged out of the recent financial crisis and is developing at a rate higher than 8.7% annually. China is still a developing country and needs further development to improve the population's living standard and infrastructure. Also, China is a country facing a lot of challenges, including shortages of various kinds of resources, air and water pollution, CO₂ emission, and so on. Although the solution is not simple, the development of industrial biotechnology may help. Biomass is abundant and renewable. Besides satisfying requirements in daily life, biomass plays a unique role in oxygen and carbon recycling. The utilization of biomass as a raw material for the production of biofuels and a variety of important chemicals has made great progress in the past few decades both in the world and in China. Some key technologies will soon breakthrough, such as: pretreatment technology for lignocellulosic materials, high efficiency production of cellulase, discovery of new platform chemicals from biomass, high-throughput screening technology for microorganisms, application of genetic engineering to industrial microorganisms, and so on. In recent years, China has been catching up in the development of industrial biotechnology. Apart from a continuously expanded production scale, new products and new technologies are under development and are transferring into commercial production quickly.

The Chinese government is firmly supporting basic and applied research in industrial biotechnology through the National Natural Science Foundation Projects 973 and 863 at a previously unseen scale. The enterprises in China are eager to develop new products and new technologies, either by themselves or in cooperation with Research Institutes and Universities. Scientists have accumulated a great deal of experience during the past few decades and created various bioprocesses in China. With an emphasis on Intellectual Right Protection, more international cooperation treaties are under discussion and more foreign companies are investing heavily in industrial biotechnology in China. The solid research background, enormous human resources and sufficient financial support will guarantee the fast growth of industrial biotechnology in China in the future. The bioprocesses will contribute more and more to sustainable development of China.

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