ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 4 (1): 1-6, 2005
ISSN 1682-297X

Development of Bioprocess Engineering in China

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Abstract: Bioprocess engineering in China embraces the old and new technologies. Before the 1980's, bioprocess engineering was focused on the development of conventional fermentation products such as wine, beer, alcohol, citric acid, antibiotics and amino acids. Bioprocess engineering started to develop rapidly since the early 1980's. Recombinant DNA technology has now been applied to the various bioprocesses to enhance the productivity and/or extend the range of new products including drugs, foods and new materials. Also various types of bioreactors and automatic bioprocesses have been developed with advanced biosensor and computer systems. Since 1996, marine biotechnology has been incorporated in the national '863' plan (i.e., National High Tech Research and Development Program), which leads to the rapid development in marine bioprocess engineering, especially in the mass production of microalgal products. Processes for the production of several high-value algal products such as Docosahexaenoic Acid (DHA), Eicosapentaenoic Acid (EPA) and astaxanthin have been developed. There are now over 400 research institutions undertaking biotechnology/ bioprocess engineering research and development. Many big companies including the Cheung Kong Groups have founded biotechnology branches.

Key words: Bioprocess engineering, fermentation, bioreactor design, marine biotechnology, research institutions, China

INTRODUCTION

China is probably one of the first countries to apply microbial fermentation to food processing and production. Examples include fermented bean curd (furu), fermented soybeans, soy sauce, vinegar, fermented fish sauce, etc. which were already used by the Chinese in ancient times. There is evidence, for instance, that fermented soybeans were used in China as early as in 200 B.C.[1]. Even now, many Chinese-style fermented foods are still very popular in the Chinese community all over the world.

Nevertheless, the concept "bioprocess engineering" is relatively new in China. Bioprocess engineering refers to the design and implementation of processes based upon living systems by using engineering principles. Bioprocess engineering in China actually embraces the old and new technologies. Before the 1980's, bioprocess engineering was focused mainly on the development of the various conventional fermentation processes for the production of food and pharmaceutical products such as wine, beer, alcohol, citric acid, enzymes, single cell protein, antibiotics and amino acids [e.g., monosodium glutamate (MSG)]. In 1978 at the First National Science and Technology Congress, biotechnology was first proposed as one of the eight important new technology fields and subsequently was included in the "National Science and Technology Development Plan for the years 1978-1985". Starting in 1981. The Ministry of Science and Technology of China has included biotechnology in the national 5-year plans[2]. Table 1 lists biotechnology research areas included in the national 5-year plans.

In 1987, in the first national "863" plan (i.e., the National High Tech. Research and Development Program with funding of 10 billion Chinese yuan, equivalent to 1.21 billion US dollars), biotechnology was ranked the first among the 7 fields identified for support[2]. As a result, the Chinese biotechnology industry has developed very rapidly in the past 20 years. In addition

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Table 1: Biotechnology research areas in the national 5-year plans

Plans and periods	Research fields	Number of topics	Projects supported
National sixth five-year plan (1981-1985)	Genetic engineering, enzyme engineering, fermentation engineering	3	Not known
National seventh five-year plan (1986-1990)	Genetic engineering and products, plant genetic engineering, cell engineering, enzyme engineering, fermentation engineering, bioreactor engineering	8	97
National eighth five-year plan (1991-1995)	Genetic engineering and drugs development, animal and plant genetic engineering, animal and plant cell engineering, enzyme engineering, biochemical engineering, marine bioengineering, fermentation engineering, development of biopharmaceuticals	12	130

Table 2: Recent conventional bioprocess engineering products and yields in China[a]

Products	Annual yield (tones)	Ranking in the world
Beer	18,880,000	Second
White spirit	7,810,000	
Soy sauce	3,600,000	
Vinegar	1,500,000	
Alcohol	2,130,000	
Yellow rice wine	1,210,000	
Fruit wine	600,000	
Grape wine	300,000	
MSG	630,000	First
Citric acid	198,000	Second
Yeast	64,000	

[a]The total value of these products output accounts for some 20% of the total value of the Chinese food industrial output

to the conventional fermentation products, there have been many new biotechnological products commercialized through the development of bioprocess engineering. These products include medical products (e.g., monoclonal antibody, diagnostic kits, drugs, vaccines, etc.), bio-pesticides, bio-fertilizers, recombinant DNA products, nutraceuticals and functional food ingredients[3]. The sale of these new products in 1996 alone reached 11.4 billion Chinese yuan, which is 50 fold that a decade ago. In 1990, there were only 274 research institutions involved in research and development of biotechnological products. In 2000, the number of research institutions undertaking biotechnology R and D increased to over 400. In Hong Kong, the Innovation and Technology Commission of the Government of the Hong Kong Special Administrative Region has set up an Innovation Technology Fund (with 5 billion Hong Kong dollars) to support technological development of which biotechnology/ bioprocess engineering is one of the major areas.

Development of bioprocess engineering

Conventional bioprocesses and products: There have been numerous bioprocesses developed to produce conventional food products by using microorganisms or enzymes. China has produced a wide spectrum of food products and food additives. The annual production of monosodium glutamate (MSG) has reached 630,000 tons, which is the highest in the world. The productions of beer and citric acid are 18,880,000 tons and 198,000 tons, respectively which are the second highest in the world. Table 2 shows the recent conventional bioprocess engineering products and yields in China[3].

Technologically, in beer production, owing to the enhanced understanding of the physiology of barley, the germination time has been reduced to 4 days. With the improvement of yeast characteristics and fermentation technology, the fermentation period for beer is now reduced to approximately 2 weeks[3].

Also new microbial strains have been isolated and used in fermentation, particularly for MSG and citric acid production. As a result, higher yield (over 10% increase) and short fermentation time (reduced by over 10%) have been achieved. In addition, larger size fermenters have been adopted which further reduce the operation costs. For instance, the fermenter vessel sizes now used are 660 m^3 and 220 m^3 for MSG and citric acid, respectively[3].

In addition to the bioprocess engineering products listed in Table 2, there are also other important bioprocesses that have been developed to produce products including certain organic acids, (e.g., L-lactic acid, L-malic acid, etc.), enzymes (e.g., high temperature α-amylase, pectinase, glucose isomerase, β-glucanase, alkaline lipase, cellulase, low-temperature alkaline protease, etc.), amino acids (e.g., lysine, L-isoleucine, leucine, valine, L-arginine, L-phenylalanine, tryptophan, etc.)[4,5]. All these products have found applications in the brewery, animal feed and the other food industries.

Bioprocess engineering has also been widely used in the pharmaceutical industry. Vitamin C (i.e., L-ascorbic acid) production is one of the examples. The fermentation of L-ascorbic acid is a 2-step processes; sorbitol is first used as raw material, which is then converted to sorbose and eventually converted to 2-keto-L-gulonic acid by microbial mixed cultures[6]. More recently, a bacterium called *Bdellovibrio* sp. has been isolated and used for vitamin C fermentation by Shenyang Institute of Applied Ecology, Chinese Academy of Sciences. As a result, the yield of 2-keto-L-gulonic acid in a 15 m^3 fermenter reaches

Table 3: Recombinant DNA drugs developed and manufactured in China[5]

Products	Diseases treated	Companies or institutes involved	Date approved
Recombinant human interferon α1b (rHuIFNα1b) (for external use)	Virus corneitis	Chang Chun Institute of Biologicals	1989
Recombinant human interferon α1b (rHuIFNα1b)	HBV, HCV	Shanghai Institute of Biologicals, Shenzhen Ke Xing Co.	1996
Recombinant human interferon α2a (rHuIFNα2a)	Herpes, HBV, HCV	Chang Chun Institute of Biologicals, Chang Shan Pharmaceutical Ltd., Shenyang Shan Sheng Pharmaceutical Co., Liaoning Institute of Biologicals, Xing Da Zhou Pharmaceutical Co.	1996 1997
Recombinant human interferon α2b (rHuIFNα2b)	HBV, HCV	Li Ya Ha Er Co., Hua Er Da Co., An Ke Biologcal Co., Zhijiang Han Sheng Pharmaceutical Co., Hua Xing Biological Co.	1996 1997
Recombinant human interferon γ(rHuIFNγ)	Rheumatics	Shanghai Ke Long Co., Shanghai Institute of Biochemistry, Li Zhu Bioengineering Co.	1994 1995
Recombinant human interleukin-2 (rHuIL-2)	Cancers	Jiangsu Jin Si Li Pharmaceutical Co., Chang Chun Institute of Biologicals, Chang Shan Pharmaceutical Ltd., Shan Sheng Pharmaceutical Co., Hua Xing Biological Co., Shengzhen Ke Xing Co., Si Huan Pharmaceutical Co.	1995 1997
Recombinant human granulocyte colony-stimulating factor (rHuG-CSF)	Leukopenia	Hanzhou Jiu Yuan Gene Co., Bei Hai Fang Zhou Pharmaceutical Co., Hua Bei Pharmaceutical Co., Jin Sai Pharmaceutical Co., Bei Lu Yuan Pharmaceutical Co., Suzhou Zhong Kai Pharmaceutical Co.	1996
Recombinant human granulocyte-macrophage colony-stimulating factor (rHuGM-CSF)	Leukopenia	Hua Bei Pharmaceutical Co., Shunde Nan Fang Pharmaceutical Co., Li Ya Ha Er Co., Hai Kou Pharmaceutical Co., Xiamen Te Bao Pharmaceutical Co	1997
Recombinant streptokinase (rSK)	Heart diseases	Yi Da Co.	1996
Recombinant human erythropoietin (rHuEPO)	Anemia	Hua Xing Biopharmaceutical Co., Shan Sheng Pharmaceutical Co., Shandong Sai Rua Jin Pharmaceutical Co., Ar Hua Biopharmaceutical Co., Fu Xing Bioengineering Co., Chengdu Di Ao Pharmaceutical Co.	1997
Basic fibroblast growth factor (bFGF)(for external use)	Wounds, Burn	Zhuhai Dong Da Group	1996
Recombinant human epidermal growth factor (rHuEGF)	Wounds, Burn	Shanghai Da Jiang Co.	1997

Table 4: Heterotrophic microalgal cultivation processes

Algae	Applications	Cell density (productivity)	Scale (system)	Organic carbon used
Chlorella protothecoides	Health food, lutein (an commercially important carotenoid)	46 g L^{-1} (11 g/L/d)	30 L, 4 m^3 (fed-batch system)	Glucose
Chlamydomonas reinhardtii	Genetically important green algae, feed for aquatic animals	9 g L^{-1} (2 g/L/d)	1 and 30 L (hollow fiber cell recycle system)	Acetate
Crypthecodinium cohnii	Docosahexaenoic acid (DHA) production, nutrient food ingredients for infants	20 g L^{-1} (7 g/L/d)	16 L (fed-batch system)	Glucose
Nitzschia laevis	Eicosapentaenoic acid (EPA) production, useful for prevention of cardiovascular diseases	40 g L^{-1} (3 g/L/d)	3.7 L (fed-perfusion systems)	Glucose
Spirulina platensis	Health food, phycocyanins and γ-linolenic acid	10 g L^{-1} (1 g/L/d)	16 L (fed-batch mixotrophic culture)	Glucose
Haematococcus pluvialis	Astaxanthin, a pink ketocarotenoid pigment and strong antioxidant, for aquaculture and as health food	3 g L^{-1} (0.5 g/L/d)	3.7 L (fed-batch system)	Acetate
Chlorococcum species	Astaxanthin	12 g L^{-1} (1.4 g/L/d)	3.7 L (batch mixotrophic culture)	Glucose
Algae-like microorganism	Docosahexaenoic acid (DHA) production, nutrient food ingredients for infants	12 g L^{-1} (9.6 g/L/d)	10 m^3 (batch culture)	Glucose

122 g L^{-1} which is probably the highest so far reported[6]. There are some 12 antibiotics that have been developed and produced by fermentation technology[5].

Recombinant DNA technology and products: Recombinant DNA technology has made a great impact in the commercial world. Recombinant DNA technology has been applied to the various bioprocesses to enhance the productivity and /or extend the range of biotechnological products. The major impact of recombinant DNA techniques is in the pharmaceutical industry although it has also been applied to the food and agriculture fields.

In 1989, the first recombinant DNA drug, recombinant human interferon α-1b (rHuIFN α-1b) was developed and marketed in China[5]. Since then, over 10 biopharmaceuticals and vaccines resulting from recombinant DNA technology have been manufactured. The annual market is approximately 1.8 billion Chinese

Table 5: Some of the novel bioreactor systems developed in China[5]

Categories	Bioreactor systems
Animal cell culture bioreactors	1) Air-lift and stir-tank bioreactor 2) Hollow-fiber cell cultivation system 3) Continuous-injection cultivation system
High viscosity culture bioreactors	1) Microbial polysaccharide air-lift bioreactor 2) PHB fermentation air-lift bioreactor
Immobilized cell bioreactors	1) External-circulation fluidized bed bioreactor 2) Spray-circulation moving bed bioreactor 3) Suspended bed bioreactor 4) Shaken fluidized bed bioreactor
Immobilized enzyme bioreactors	1) Membrane bioreactor 2) Micro-carrier bioreactor
Large-scale fermentation bioreactors	1) Double-circulation air-lift bioreactor (100 and 300 m^3) 2) Solid state bioreactor
Plant cell culture bioreactors	1) Air-lift circulation plant cell bioreactor (30 and 200 L) 2) Semi-submerged tissue culture bioreactor (40 L) 3) Multi-layers tissue culture bioreactor
Photobioreactors	1) Algal air-lift photobioreactor 2) Algal continuous separation bioreactor
Standard type automatic bioreactors	From 2.5 to 300 L, suitable for laboratory or pilot scale cultivation experiments

yuan in 1996. It is estimated that the market may exceed 5 billion Chinese yuan in 2001. In addition, over 10 additional recombinant DNA drugs have been approved in clinical trials and many more are in the stage of pre-clinical studies[5]. Table 3 lists those recombinant DNA drugs developed and manufactured in China.

Marine biotechnology and products: In 1996, marine biotechnology was formally included in the national '863' plan (High Tech. Research and Development Program)[5]. As a result, research and development in aquacultural biotechnology has progressed very rapidly. Bioprocess engineering has been applied to the development of cultivation of microalgae, particularly for the production of health foods and functional ingredients from mass culture of *Spirulina* (blue-green algae or cyanobacteria), *Chlorella* (green algae) and *Dunalialla* (green algae). For *Spirulina* alone, currently there have been more than 80 production companies in China, with a total annual production of more than 350 t (dry powder) and total production area of over 1,000,000 m^2 by using open pond photosynthetic cultivation strategies[7]. In northern China, some cultivation ponds are modified and covered with transparent plastic sheet to raise culture temperature (particularly in winter) and also help minimize chances of contamination.

Photosynthesis is known as the most efficient life supporting process on earth. For this reason, at present, most algal industrial processes are based on open pond technology in which light is required as energy source, but the inability to control environmental factors is the major unsolved problem, which limit its development. An alternative approach is to develop heterotrophic or mixotrophic culture technology, which employs the extra capacity of present fermentation industries[8]. The heterotrophic strategy can on one hand avoid contamination and light limitation and on the other hand improve the yield and quality of products by perfectly controlling the nutritional and environmental conditions. The University of Hong Kong has successfully developed a few heterotrophic and mixotrophic culture strategies for the cultivation of a number of commercially important microalgae at high cell densities including *Chlorella protothecoides*[9], *Chlamydomonas reinhardtii*[10], *Crypthecodinium cohnii*[11], *Nitzschia laevis*[12,13], *Spirulina platensis*[14], *Haematococcus pluvialis*[15], *Chlorococcum* species [16] and *Chlorella zofingiensis*[17] Table 4 shows the microalgae and heterotrophic or mixotrophic cultivation processes developed in Hong Kong, in collaboration with industries and universities in the mainland of China.

Bioreactor designs and process monitor and control: Bioreactor designs and process monitor and control are an important part in bioprocess engineering. For the last 20 years, many novel bioreactor systems have been developed some of which have been applied to large-scale industrial fermentation. Table 5 summarizes some of the bioreactor systems developed in China.

Bioprocess monitor and control require development of advanced biosensors and computer on-line control systems. For the last 10 years, a number of biosensors have been developed and applied successfully to industrial production. These include pH biosensor (autoclavable), foam biosensor and controller (autoclavable), CO_2 biosensor (autoclavable), dissolved oxygen biosensor (autoclavable), cell concentration biosensor (autoclavable), glucose biosensor, fermented sugar biosensors, urea biosensor and glutamate biosensor[18].

In addition, a number of on-line control and optimization systems have been developed and used in industrial fermentation including penicillin fermentation (leading to a 22% increase in yield), glutamic acid fermentation (a 11% increase) and erythromycin fermentation (a 50% increase)[5].

Research institutions and support: Since the early 1980's, biotechnology, particularly bioprocess engineering has gained great support from the Chinese government, research institutions and industries. Many funding schemes (e.g., Biotechnology Key Projects Scheme within the National 5 Year Plan, '863' High Tech. Research and Development Program, Spark Plan, Torch Plan, etc.) have been established to support biotechnology research projects. Two state key laboratories, namely, State Key Laboratory of Biochemical Engineering (in Beijing) and State Key Laboratory of Bioreactor Engineering (in Shanghai) were founded[18]. In 1996, the Ministry of Science and Technology of China supported the establishment of the National Biochemical Engineering Research Center. The center serves to incubate biotechnology projects and eventually transfer the technologies to industry. Projects conducted in the center cover almost all aspects of bioprocess engineering. There are now 4 sub-branches established which are located, respectively in Beijing, Shanghai, Nanjing and Shenzhen. The Center has its own pilot plants and scale-up facilities. Up to now, over 10 projects have been completed and successfully transferred to industry[18].

Nationwide, there are over 400 research institutions involved in bioprocess engineering and related research. They mainly belong to the Chinese academy of sciences (CAS) and the various universities. The CAS has set up a Biotechnology Experts Committee since 1987 consisting of the renowned biotechnologists from the various institutes of the CAS. The key institutes involved include Institute of Microbiology, Institute of Biophysics, Institute of Genetics, Institute of Botany, Institute of Developmental Biology, Institute of Process Engineering, Shanghai Institute of Biochemistry, Shanghai Institute of Cell Biology, Shanghai Institute of Plant Physiology, Shanghai Institute of Organic Chemistry, Shanghai Bioengineering Research Center, Shanghai Institute of Pharmacy, Chengdu Institute of Biology, Institute of Hydrobiology, Wuhan Institute of Virus, Qingdao Institute of Oceanology, Shenyang Institute of Applied Ecology, Dalian Institute of Chemical Physics, South China Institute of Botany, Institute of Zoology, Kunming Institute of Zoology, Kunming Institute of Botany, etc[19]. In addition, almost all key universities (including several universities in Hong Kong) have research programs in biotechnology and bioprocess engineering.

Although biotechnology/bioprocess engineering has developed very rapidly in China, especially since the early 1980's, research in this field still falls behind those of the developed countries. China has now joined WTO and many other international organizations and activities. There is a need and desire for Chinese biotechnologists to collaborate with their counterparts abroad. It can be foreseen, with the strong support from the Chinese government, research institutes, universities and industries, the development of Chinese biotechnology/bioprocess engineering will be able to reach to a new level before long.

ACKNOWLEDGMENT

The authors gratefully acknowledge the support of the Innovation and Technology Commission of the Government of the Hong Kong Special Administrative Region for this work.

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