DESIGN PROJECT FOR PRODUCTION OF IFN-ALPHA

CHE 496 IPRO: SPRING 2006

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TABLE OF CONTENTS

ABSTRACT

The IPRO goal was to design a process for the production of the biotherapeutic compound IFN-Alpha from Chinese Hamster ovaries. After the process design was complete, a process flow diagram showing the actual process and separation steps was created and finally an economic analysis of the market feasibility of the product was prepared. The approach to the IPRO involved dividing the objectives into three major groups: (1) the Che 496 group was involved in the process design and the separation processes; (2) the Che 296 group was involved in the public perception of biotechnology, the useful products from biotechnology and the survey of the commercial biotherapeutic compounds; and (3) the 435 group was involved with the history of biotherapeutics, classification and actions infernos and the companies involved in the production of therapeutics. The individual members of these groups are seen on the team list below.

IPRO OBJECTIVES

The objective of this IPRO was to design the production process (including flow diagram and economic analysis) of the IFN-Alpha protein – an antiviral glycoprotein – from Chinese Hamster ovarian cells. Through the design of the process and the profitability and cost analysis, the IPRO group will be able to assess whether the production of this biotherapeutic compound is economically feasible, profitable and determine if there is a large and continuing demand for IFN-Alpha on the current market.

HISTORY OF BIOTECHNOLOGY

Biotechnology is defined as the use of a living organism to make a product or run a process. Although people tend to think of biotechnology as a new economic sector, its roots are traceable back to over 6,000 years to the time when beer was first fermented. Indeed, early biotechnology was almost exclusively focused on fermentation techniques to produce drinks, food and fuel. The word biotechnology itself can be traced to 1917, when it was used to refer to a large-scale production of materials from microbes grown in vats. Narrow definitions often limit biotechnology to genetic engineering and recombinant DNA technology. Genetic engineering became a reality when a man-made gene was used to manufacture a human protein in a bacteria for the first time. Biotech companies and universities were off to the races, and the world would never be the same again. In 1978, in the laboratory of Herbert Boyer at the University of California at San Francisco, a synthetic version of the human insulin gene was constructed and inserted into the bacterium *Escheria coli*. Since that key moment, the trickle of biotechnological developments has become a torrent of diagnostic and therapeutic tools, accompanied by ever faster and more powerful DNA sequencing and cloning techniques.

Biotechnology seems to be leading a sudden new biological revolution. It has brought us to the brink of a world of "engineered" products that are based in the natural world rather than on chemical and industrial processes. Biotechnology has been described as "Janus-faced". This implies that there are two sides. On one, techniques allow DNA to be manipulated to move genes from one organism to another. On the other, it involves relatively new technologies whose consequences are untested and should be met with caution. The term biotechnology was coined in 1919 by Karl Ereky, an Hungarian engineer. At that time, the term meant all the lines of work by which products are produced from raw materials with the aid of living organisms. Ereky envisioned a biochemical age similar to the stone and iron ages. (Bud, 1989)

A common misconception is that biotechnology only includes DNA and genetic engineering and current knowledge of biotechnology suggests emphasis on the techniques of DNA science as the "end-and-all" of biotechnology. Biotechnology is NOT new. Man has been manipulating living things to solve problems and improve his way of life for millennia.

Early agriculture concentrated on producing food. Plants and animals were selectively bred and microorganisms were used to make food items such as beverages, cheese and bread.
The late eighteenth century and the beginning of the nineteenth century saw the advent of vaccinations, crop rotation involving leguminous crops, and animal drawn machinery.

The end of the nineteenth century was a milestone of biology. Microorganisms were discovered, Mendel's work on genetics was accomplished, and institutes for investigating fermentation and other microbial processes were established by Koch, Pasteur, and Lister.

Biotechnology at the beginning of the twentieth century began to bring industry and agriculture together. During World War I, fermentation processes were developed that produced acetone from starch and paint solvents for the rapidly growing automobile industry. Work in the 1930s was geared toward using surplus agricultural products to supply industry instead of imports or petrochemicals. The advent of World War II brought the manufacture of penicillin. The biotechnical focus moved to pharmaceuticals. The "cold war" years were dominated by work with microorganisms in preparation for biological warfare as well as antibiotics and fermentation processes. (Goodman, 1987)

Biotechnology is currently being used in many areas including agriculture, bioremediation, food processing, and energy production. DNA fingerprinting is becoming a common practice in forensics. Similar techniques were used recently to identify the bones of the last Czar of Russia and several members of his family. Production of insulin and other medicines is accomplished through cloning of vectors that now carry the chosen gene. Immunoassays are used not only in medicine for drug level and pregnancy testing, but also by farmers to aid in detection of unsafe levels of pesticides, herbicides and toxins on crops and in animal products. These assays also provide rapid field tests for industrial chemicals in ground water, sediment,

and soil. In agriculture, genetic engineering is being used to produce plants that are resistant to insects, weeds and plant diseases.

New biotechnological techniques have permitted scientists to manipulate desired traits. Prior to the advancement of the methods of recombinant DNA, scientists were limited to the techniques of their time - cross-pollination, selective breeding, pesticides, and herbicides. Today's biotechnology has its "roots" in chemistry, physics, and biology . The explosion of the techniques have resulted in three major branches: genetic engineering, diagnostic techniques, and cell/tissue techniques. While this module contains many items involving new techniques that emphasize DNA science, the user should keep in mind that DNA manipulation is but the latest tool commonly available to biotechnologists during this revolution.

Biology and biotechnology milestones of note include:

- 8000 BC Collecting of seeds for replanting. Evidence that Mesopotamian people used selective breeding (artificial selection) practices to improve livestock.
- 6000 BC Brewing beer, fermenting wine, baking bread with help of yeast
- 4000 BC Classical biotechnology: Dairy farming develops in the Middle East; Egyptians use yeasts to bake leavened bread and to make wine.
- 2000 BC Egyptians, Sumerians and Chinese develop techniques of fermentation, brewing and cheese-making.
- 1500 Acidic cooking techniques lead to sauerkraut and yogurt - two examples of using beneficial bacteria to flavor and preserve food. Aztecs make cakes from Spirulina algae.
- 1590 The microscope is invented by Zacharias Janssen.
- 1675 Microorganisms discovered (using first microscope)
- 1856 Gregor Mendel discovered the laws of inheritance
- 1859 *On the Origin of Species* - English naturalist Charles Darwin's theory of evolution - is published in London.
- 1861 French chemist Louis Pasteur develops pasteurization - preserving food by heating it to destroy harmful microbes.
- 1865 Austrian botanist and monk Gregor Mendel describes his experiments in heredity, founding the field of genetics.
- 1879 William James Beal develops the first experimental hybrid corn.
- 1910 American biologist Thomas Hunt Morgan discovers that genes are located on chromosomes.
- 1914 Gerry FitzGerald's development and production of Canada's first diptheria antitoxin lead to the establishment of the University of Toronto Antitoxin Laboratories, later renamed the Connaught Laboratories. The labs now serve as a division of Aventis Pasteur the world's largest producer of vaccines.
- 1919 Karl Ereky, a Hungarian agricultural engineer, first used the word biotechnology
- 1921 discovery of insulin at the University of Toronto by Banting, Best, Collip and MacLeod.
- 1922 Development and use of insulin in the treatment of diabetes.
- 1928 F. Griffith discovers genetic transformation - genes can transfer from one strain of bacteria to another.

Modern biotechnology or second generation biotechnology grew out of molecular biology and genetic engineering and emerged after World War II. It involved the integration of microbiology, biochemistry and chemical engineering for large-scale fermentation, sewage treatment, and for applications in the chemical and pharmaceutical industries, is in its early stages.

- 1941 Danish microbiologist A. Jost coins the term genetic engineering in a lecture on sexual reproduction in yeast.
- 1943 Oswald Avery, Colin MacLead and Maclyn McCarty use bacteria to show that DNA carries the cell's genetic information.

- 1953 James Watson and Francis Crick describe the double helix of DNA, using x-ray diffraction patterns of Rosalind Franklin and Maurice Wilkins.
- 1960's Olah Hornykiewicz, who originally discovered that Parkinson's disease patients had less dopamine in their brains, continued to contribute to the development of L-Dopa as a therapeutic agent while working in Toronto.
- 1961 Discovery of the hematopoietic stem cell by Toronto researchers.
- Early 1970's Paul Berg, Stanley Cohen and Herbert Boyer develop ways to cut and splice DNA, introducing recombinant DNA techniques.
- 1972 The DNA composition of humans is discovered to be 99% similar to that of chimpanzees and gorillas.
- 1973 Breakthrough discovery of recombinant DNA became the platform for research in cloning, genomics and proteomics.
- 1974 Discovery of P-glycoprotein by Toronto researchers.
- 1975 Scientists organize the Asilomar conference to discuss regulating recombinant DNA experiments. George Kohler and Cesar Milstein show that fusing cells can generate monoclonal antibodies.
- 1980 Modern biotech is characterized by recombinant DNA technology. The prokaryote model, *E. coli*, is used to produce insulin and other medicine, in human form. (About 5% of diabetics are allergic to animal insulins available before)
- 1982 First genetically engineered product - human insulin produced by Eli Lilly and Company using E. coli bacteria - is approved for use by diabetics.
- 1983 Discovery by Toronto researchers of the T-cell receptor, described as the "holy grail" of immunology.
- 1984 Kary Mullis develops polymerase chain reaction (PCR) to mass-produce specific DNA fragments.
- 1986 First release into the environment of a genetically engineered plant (a tobacco).
- 1987. First release of genetically engineered microbes in field experiments.
- 1990, the international Human Genome Project, a 13-year effort, is launched. The goals of the project were to identify and sequence all of the genes in the human genome.
- 1994 FDA approves of the first GM food from Calgene: "Flavr Savr" tomato
- 1997 British scientists from the Roslin Institute report cloning a sheep called Dolly using DNA from two adult sheep cells. Ian Wilmut led the team that cloned Dolly.
- 2001 Due to effective resource and technological advances the Human Genome Project accelerated and a map of the entire human genome sequence with analysis was published.
- 2002 Researchers sequence the DNA of rice, the main food source for two-thirds of the world's population. Rice is the first crop to have its genome decoded.
- 2003 GloFish, the first biotech pet, hits the North American market. Specially bred to detect water pollutants, the fish glows red under black light thanks to the addition of a natural bioluminescence gene.

Scientists can now manipulate DNA, the fundamental building block of life. Early results range from the manufacture of genetically engineered drugs to the cloning of Dolly, the sheep. With the completion of the initial sequencing and draft of the human genome, the next step will be the identification of new kinds of drugs. It is expected that the number of drugs identified, tested and commercialized will increase six-fold over the next 20 years.

In addition to improved drug therapies, diseases diagnosis and treatment benefit from genomics, these new technologies may allow doctors to test individual genetic profiles against a group of drugs available for a specific condition in order to identify the most effective treatment within the next 15 to 20 years.

PUBLIC PERCEPTION OF BIOTECHNOLOGY

Within the field of biotechnology, very few subjects have escaped controversy. Genetically modified crops are not an exception and since researchers in the California Institute of Technology genetically modified maize in the early 1990s, the world has been forced to consider if genetically modified crops should be introduced into the food supply and how genetically modified foods should be regulated if introduced. According to biotechnology public opinion polls taken by the Pew Initiative on Food and Biotechnology, CNN, and the Eurobarometer, European and American people alike were optimistic about the benefits of genetically modified crops in 1990. Opposition has increased since the public's optimistic embrace of genetically modified crops in the early 1990s. Currently, the European Union is generally opposed to the sale of genetically modified foods and the growth of genetically modified crops. However, public opinion does vary throughout the European Union. When the general public considers whether genetically modified crops should be introduced into the food supply, they consider the risks and the possible gains. If the possible gains are perceived to outweigh the risks, then the public support for genetically modified crops generally increases. In 1999, news coverage of genetically modified foods peaked in Europe and in America. Many European countries changed their points of view on genetically modified crops during this year. France, Greece, and Luxembourg's support for gm foods and crops drastically decreased. This trend was seen throughout Europe and America where the safety of genetically modified crops was being called into public attention.

In the late 1990s and early 2000s, the European and American public showed little support for growing and consuming genetically modified crops due to the modest perception of the possible gains involved in consuming and growing genetically modified crops (Eurobarometer and pewagbiotech.org). The Europeans generally distrusted those involved in the industry when they presented information concerning genetically modified foods and also generally distrusted their government regulators in protecting the general public from the possible hazards of genetically modified foods. Many factors contributed to this mistrust, two major factors affecting public perception were the changes taking place in European government and the mad cow disease outbreak (Eurobarometer and CNN). On the American side, support for growing genetically modified crops has barely increased during this time. However, less and less Americans answered that they would buy genetically modified foods. American perception of the safety of genetically modified foods decreased as well.

In the early 2000s, the trends in European public opinion changed. The public began to trust researchers and government food regulators more. The scientific community made a solid effort to push the possible benefits of genetically modified crops such as low pesticide use, higher yields of food produced, cheaper food, and food that stays fresher longer into the public eye. While Europeans generally oppose introducing gm foods into the food supply and growing gm crops, the opposition began to shrink in the early 2000s and support began to increase in the early 2000s (Eurobarometer). However, the majority of Europeans said that they would be unlikely to buy genetically modified foods soon in recent polls. Less than 10% of Europeans said that they would be likely to buy genetically modified foods in recent polls.

According to the Pew Initiative on Food and Biotechnology, a strong majority of Americans believe that genetically modifying food could produce good results in the future. This study also indicated that a strong majority of Americans support continued research into this new technology. However, America is evenly divided upon the belief that genetically modified foods are safe or unsafe. However, Americans currently resist buying genetically modified foods because they do not have full confidence in the safety of the new food. According to a 2005 ABC news poll, 55% of American consumers are unlikely to buy genetically modified foods soon, while only 8% of Americans said that they are likely to buy genetically modified foods soon.

USEFUL PRODUCTS FROM BIOTECHNOLOGY

MEDICINE

Variations in genetic makeup are responsible for an estimated 5,000 hereditary disorders including Huntington's disease, cystic fibrosis and sickle cell anemia. Developments in biotechnology (including the discovery of genes, new techniques and advances in current medical knowledge) could lead to treatments for presently untreatable conditions and potentially eliminate the need for organ donation. Advances in biotechnology will revolutionize medical treatment, moving from diagnostics and treatment to detection and prevention. The announcement of the first working draft of the human genome marks a significant step forward in our understanding of the way in which we understand and develop treatments for incurable genetic conditions. The human genome project has not only enabled us to identify the vital role that genes play in human disease and illness, but to accelerate these discoveries.

In conclusion, biotechnology contributed:

- New drugs, antibiotics and vaccines to prevent disease
- Repair of damaged organs and tissues and improved detection of diseases;
- Treatments for human infertility
- Genetic therapy
- Viruses, and bacteria in-depth study
- Bioinformatics

FOOD AND AGRICULTURE

In agriculture, the crops are genetically modified to the following standards:

- herbicide tolerant
- pest resistant
- virus resistant
- stacked trait
- safe for human/animal consumption

73 of biotech crops have been approved for human/animal consumption in North America. 56 in US, 54 in Canada and 3 in Mexico.

Biotechnology applied major crops

Biotech canola, corn, cotton and soybeans accounted for more than 99 percent of worldwide biotechnology crops in 2001. In 2001 in the United States alone, these four crops plus biotech papaya and squash produced an additional 4 billion pounds of food and fiber, increased farm income by $1.5 billion and reduced pesticide use by 46 million pounds.

Food Science

Fermented products: Cheese, wine, brewing beers

Bacteria studies and modification are utilized in designing food preservatives or other protective processes. Agricultural biotechnology has been hindered by controversies over genetic modification, which have led to consumer and government resistance despite its potential to boost agricultural yields in poorer countries

INDUSTRY AND ENVIRONMENT

A. Bio industry: The Bio-industry is a new engineering field to industrialize biotechnology and has made significant advances in our understanding of the way in which plants, animals and humans work, with direct benefits in many areas including healthcare, diagnostics, environmental and agricultural biotechnology and a growing range of non-traditional industrial applications.

Environmental Treatment: Biotechnology is used to recycle, treat waste, clean up sites contaminated by industrial activities (bioremediation). Examples:

B. Environmental Treatment: Biotechnology is used to recycle, treat waste, clean up sites contaminated by industrial activities. Examples include

Palm Oil - Environmental Management for Palm Oil Mill

Palm Oil Production: Palm oil mills with wet milling process are accounted for major production of palm oil in Malaysia, Indonesia and Thailand. Beside the main product "crude palm oil" the mills generate many by-products and liquid wastes which may have a significant impact on the environment if they are not dealt properly. One ton of fresh fruit bunches (FFB) composed of 230-250 kg of empty fruit bunch (EFB), 130-150 kg of fibers, 60-65 kg of shell and 55-60 kg of kernels and 160-200 kg of crude oil. EFB are bulk solid residues.

Palm Usage: The use of EFB as a fuel for boiler is constrained by its high moisture content and low heating value (dry EFB <10 MJ/kg). Utilization of the EFB as substrate for mushroom cultivation and for the production of particleboard should be given first priority. In addition EFB could be used as organic fertilizer and mulching material. Palm fibers are used mainly as fuel for boilers (heating value of dry fibers <5 MJ/kg). Other applications of palm fibers include its use as substrate for enzymatic saccharification as animal feed. Although, palm shell can be used as boiler fuel with heating value of 17 MJ/kg, it causes the black smoke. Alternative use for the production of activated carbon is preferable. Decanter cake can be used as a fertilizer or soil conditioner.

Problem & Solution: Palm oil mill effluent (POME) composed of high polluted effluent (from sterilizer and oil room) and low polluted effluent (steam condensate, cooling water, boiler discharge and sanitary effluent). To minimize overall treatment costs, the different wastewater streams should be collected and treated separately. The oil separation from the wastewater stream by gravity type oil separators is recommended which will contribute to improve production yield and minimize the organic loading for the subsequent biological treatment system. The most appropriate secondary treatment for POME is biological digestion in which the combination of anaerobic and aerobic ponds is used presently. Closed anaerobic system should be used for energy conservation. Application of biologically treated POME for irrigation has been carried out by some palm oil mills having their own plantation nearby. The POME dosage should be based on the fertilizer requirement of plants. Spillage of POME into ground water or into surface water must be avoided. If these wastes are properly managed as described above, the palm oil mill will become an environmental0 friendly industry.

BIOFUELS

Biofuels are renewable energy sources from living things; they are to be distinguished from fossil fuels which are also of biological origin but which are non-renewable. Gas or liquid fuel made from plant material (biomass) include wood, wood waste, wood liquors, peat, railroad ties, wood sludge, spent sulfite liquors, agricultural waste, straw, tires, fish oils, tall oil, sludge waste, waste alcohol, municipal solid waste, landfill gases, other waste, and ethanol blended into motor gasoline. Biofuel is any gas or liquid fuel that derives either from recently living organisms or their metabolic byproducts, such as manure from cows.

A. Bioalcohols: Ethanol (not produced from petroleum) - A significant amount of ethanol produced from sugar beets is being used as automotive fuel in Brazil. Ethanol produced from corn (maize) is being used as a gasoline additive (oxygenator) in the United States.

B. Lipid Biofuels: SVO - used in modified diesel engines.Biodiesel - obtained from animal fats and vegetable oil (vegoils), using trans-esterification. Properly processed biodiesel is satisfactory for use in diesel cars.

C. Gas: Methane - Methane produced by the nature decay of garbage at garbage dumps is collected and used in place of fossil methane. Methane from manure decay is sometimes collected for use as fuel.

SURVEY OF COMMERCIAL BIOTHERAPEUTIC COMPOUNDS

Introduction

There are nearly 500 biotechnology products approved or in development globally, and with production capacity limited, the need for efficient means of therapeutic protein production is apparent. Through genetic engineering, plants can now be used to produce pharmacologically active proteins, including mammalian antibodies, blood product substitutes, vaccines, hormones, cytokines, and a variety of other therapeutic agents. Efficient biopharmaceutical production in plants involves the proper selection of host plant and gene expression system, including a decision as to whether a food crop or a non-food crop is more appropriate. Product safety issues relevant to patients, pharmaceutical workers, and the general public must be addressed, and proper regulation and regulatory oversight must be in place prior to commercial plant-based biopharmaceutical production. Plant production of pharmaceuticals holds great potential, and may become an important production system for a variety of new biopharmaceutical products.

Approved Agent's list

The following biopharmaceutical agents were taken from the list of agents that have been approved by the Food and Drug Administration for marketing in the United States. The trademarked name is followed by the generic name of the drug in parentheses.

Acctimmune (gamma interferon)

Genentech, Inc.
Approved for use in the management of chronic granulomatous disease (December, 1990).

Intron A (alpha-interferon)

Schering-Plough Corp.
Approved for the treatment of hairy cell leukemia (June, 1986), genital warts (June, 1988), AIDS-related Kaposi's sarcoma (November, 1988), non-A, non-B hepatitis (February, 1991), and hepatitis B (July, 1992).

Procrit (epoetin alfa)

Ortho Biotech
Approved for use in the treatment of anemia associated with chronic renal failure (December, 1990) and anemia in Retrovir-treated, HIV-infected patients and chemotherapy-associated anemia (April, 1993).

Roferon-A (recombinant alfa-interferon)

Hoffman-La Roche
Approved for the treatment of hairy cell leukemia (June, 1986) and AIDS-related Kaposi's sarcoma (November, 1988).

Alpha-interferon

Intron-A and Roferon-A are trade names for Interferon Alfa. IFN-alpha and Alpha interferon are other names for Interferon Alfa. Intron-A is a "biologic response modifier", it is classified as a "cytokine". Alpha interferon, including Intron-A, cause or aggravate fatal or life-threatening neuropsychiatric, autoimmune, ischemic, and infectious disorders. Patients should be monitored closely with periodic clinical and laboratory evaluations. Patients with persistently severe or worsening signs or symptoms of these conditions should be withdrawn from therapy. In many but not all cases these disorders resolve after stopping Intron-A therapy. All patients receiving Intron-A therapy experienced mild-to-moderate side effects. Some patients experienced more severe side effects, including neutropenia, fatigue, myalgia, headache, fever, chills, and increased SGOT. Other frequently occurring side effects were nausea, vomiting, depression, alopecia, diarrhea, and thrombocytopenia.

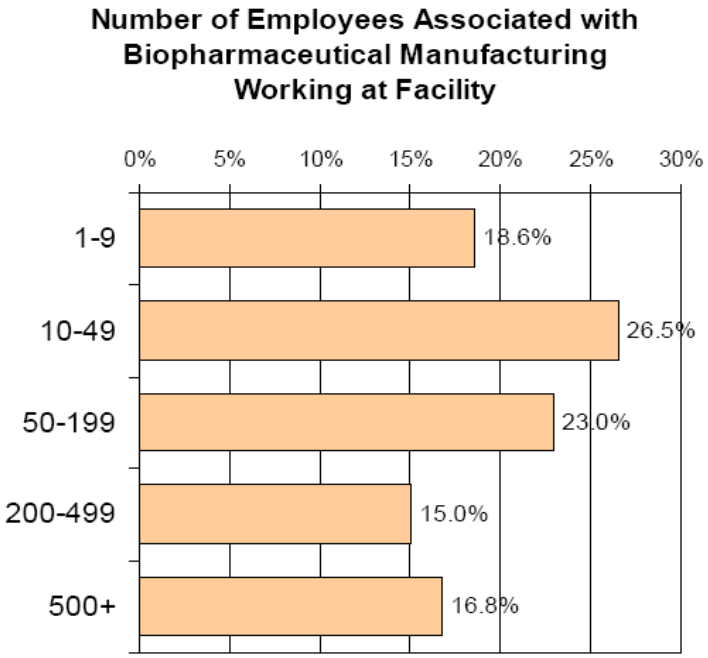
Gamma Interferon
Gamma interferon (GAM-a in-ter-FEER-on) is a synthetic (man-made) version of a substance naturally produced by cells in the body to help fight infections and tumors. Gamma interferon is used to treat chronic granulomatous disease and osteopetrosis.

Epoetin-Alfa

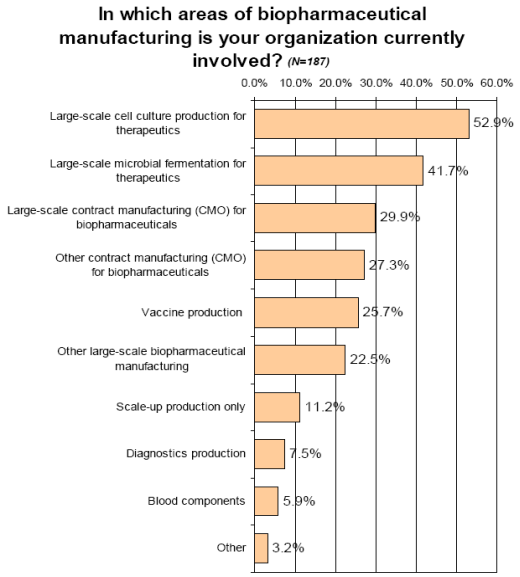
Epoetin Alfa is a man-made form of a naturally occurring protein called erythropoietin. Erythropoietin is produced in the kidney and stimulates the production of red blood cells. The amount of erythropoietin in the body may be diminished when the kidneys are damaged. Medications may also decrease the number of red blood cells.

Biopharmaceutical Manufacturing Capacity and Production:

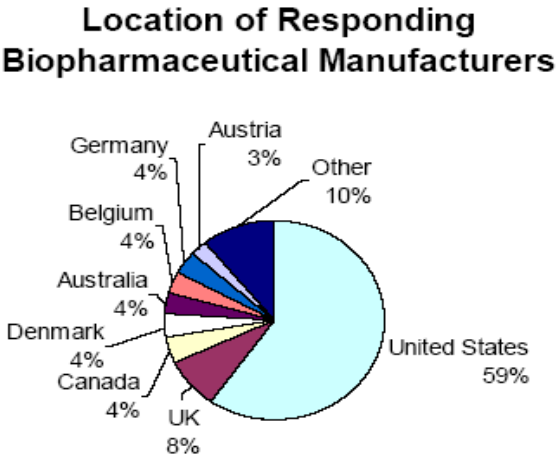
Based on survey conducted by BioPlan Associates, Inc in 2005, responsible individuals at 187 biopharmaceutical manufacturers and contract manufacturing organizations around the world have been surveyed. Using a web-based survey tool, information regarding respondents' current capacity, production, and outsourcing trends and issues were obtained and evaluated. The results are summarized by the charts below.



In terms of size of respondents' biopharmaceutical manufacturing facilities, the largest percentage of respondents (26.5%) had manufacturing staff in the 10-49 person range. Nearly 17% of respondents had over 500 employees engaged in biopharmaceutical manufacturing at their facility.



Of the 187 biopharmaceutical manufacturers and contract manufacturing organizations (CMOs) responding to the 2005 survey, 53% were involved in large-scale cell culture production; 42% were involved in large-scale microbial fermentation for therapeutics; 30% in large-scale contract manufacturing, and 27% in other contract manufacturing for biopharmaceuticals. Eleven percent was involved in scale-up production only.



Nearly 60% of respondents were from the United States, with Europe representing 29% of respondents. Other countries represented in the survey included: Cuba, Finland, France, Ireland, Israel, Italy, Portugal, Slovenia, Sweden, and Switzerland

COMPANIES INVOLVED IN PRODUCTION OF BIOTHERAPEUTICS

The healthcare sector boasts of a booming industry for biotherapeutics in recent years. Some of the traditional consumer healthcare companies have been involved in active research of anti-cancerous drugs, drugs supporting treatment of neurological, nephrological and respiratory disorders. The research involves the study of the effect of different proteins on the immune system of the human body.

The following table corresponds to those companies that produce biotherapeutic products. We should point out that due to the fact that most of these biotherapeutical companies are private, for several of them we have not been able to obtain information regarding the sales and/or income corresponding to the year 2005. For those we did, all information has been found in their respective web pages, or made available directly by a customer service representative.

COMPANY NAME	WEBSITE	MAJOR PRODUCTS	2005 SALES (MIL.)	2005 NET INCOME (MIL.)
Johnson & Johnson	http://www.jnj.com	AnemiaPro™, ARESTIN® (minocyclineHCI), LEUSTATIN® (cladribine)and genomics-based drug discovery, focusing on identification of small molecule and protein therapeutics	$50,514	N/A
Genentech Inc.	http://www.gene.com	Rituxan(anti-CD20 antibody), Herceptin(anti-HER2 antibody), Raptiva(anti-CD11a antibody), Avastin(humanized antibody),RI624(humanized monoclonal antibody), TNKase(thrombolytic agent), Activase(tissue plasminogen activator) Nutropin&Protropin(growth hormones.	$3,980	$784.8
Amgen Inc.	http://www.amgen.com	EPOGEN & Aranesp(stimulate the production of red blood cells to treat anemia), Neulasta&NEUPOGEN (selectively stimulate the production of neutrophils), and ENBREL(TNF inhibitor)	$12,430	$3,674.0
Bayer Corporation	http://www.bayerus.com	Kogenate® FS, Antihemophilic Factor (Recombinant), Formulated with Sucrose	$11,504	N/A
Applied Biosystems Group	http://www.appliedbiosystems.com	host of products and services for researching genes and proteins, pharmacokinetics and pharmacogenomics	$1,787.1	$236.9
Genzyme Corp.	http://www.genzyme.com	Cerezyme® (imiglucerase for injection), Fabrazyme® (second enzyme replacement therapy for a lysosomal storage disorder), Aldurazyme® (mucopolysaccharidosis I (MPS	$2,201.1	$86.5

		I),Hectorol® (vitamin D_2 analog indicated for the treatment ofsecondary hyperparathyroidism), Thymoglobulin® (immunosuppressive polyclonal antibody), Campath® (humanized monoclonal antibody to treat B-CLL),		
Hoffmann-La Roche Inc.	http://www.rocheusa.com	Tamiflu(influenza fighter),Accutane(controversial acne medication), Fuzeon(HIV inhibitor), Xenical(obesity drug) and Pegasys(hepatitis C therapy)	N/A	N/A
Biogen Idec Inc.	http://www.idecpharm. com	AVONEX(treatment of relapsing forms of multiple sclerosis), RITUXAN(treatment of certain B-cell non-Hodgkin's lymphomas (NHL) or B-cell NHLs, Lupus & LSE), TYSABRI(treat relapsing forms of MS to reduce the frequency of clinical relapses),ZEVALIN(treatment of cancer), and AMEVIVE(treatment for adult patients with moderate-to-severe chronic plaque psoriasis)	$2,211.6	$25.1
Chiron Corp.	http://www.chiron.com	Pediatric and adult vaccines, including influenza; meningococcal, travel, and pediatric vaccines; drugs for treatment of cancer, infectious, and pulmonary diseases which include small molecules, therapeutic proteins, and monoclonal antibodies	$1,723.4	$79.0
GTC Biotherapeutics, Inc.	http://www.transgenics.com	ATryn, a recombinant human atithrombin; rhSA, a recombinant human serum albumin; a malaria vaccine produced from transgenic mice; products that could treat rheumatoid arthritis and cancer	$4.2	$30.1
NaPro BioTherapeutics (Tapestry Pharmaceuticals)	http://www.tapestrypharma.com	new drugs for various cancers, including breast, ovarian, pancreatic, and small cell lung; anticancer agent, Paclitaxel, may be the basis for a targeted highly specific anti-tumor therapy	N/A	$17.6
CardioVascular BioTherapeutics Inc	http://www.cvge.com/	new drugs for the treatment of cardiovascular disease; protein drug candidate is CardioVascu-Grow™.	$7.9	$25.7

DOR BioPharma Inc.	http://www.dorbiopharma.com	nasally administered vaccines for such bioterror threats as ricin and botulinum toxins; orBec for therapy in intestinal graft-versus-host disease and other gastrointestinal disorders, such as Crohn's disease and ulcerative colitis.	$1	$5.9
Northwest Biotherapeutics, Inc	http://www.nwbio.com	the DCVax vaccine platform, which uses dendritic cells to program a patient's T cells to kill cancer and targeting prostate, brain, lung, and other types of cancer; the HuRx cancer therapy platform, which is based on monoclonal antibodies and does not contain any mouse proteins	$0.4	$8.5
Juvaris BioTherapeutics	http://www.juvaris.com	therapeutic vaccines for cancer and infectious diseases using its novel technology platform of cationic lipids and non-coding DNA complexes, which they claim, are capable of up-regulating the immune response, in particular cell-mediated immunity, at least 20-fold greater than existing technologies	N/A	N/A
Protiva Biotherapeutics	http://www.protivabio. com/	Focused on products to fight against cancer, metabolic and infectious disease. It's technology is based on employing lipid nanoparticles to encapsulate and deliver nucleic acid based drugs, such as siRNA, allowing for the development of molecular therapeutics that act selectively at sites of disease.	N/A	N/A
MG Biotherapeutics LLC.	http://www.mgbiotherapeutics.com	Develops treatments for heart damage resulting from myocardial infarction, and cell therapies to repair damaged heart tissue	N/A	N/A

The field of biotechnology is becoming increasingly competitive as more technology becomes available to help satisfy the ever-growing need for more products. Companies are spending billions of dollars in Research and Development expenditures trying to develop the top products. The Top 10 Biopharma rankings are annually compiled by Contract Pharma and they have long been a trusted name in assessing the biopharmaceutical company industry. Based on 2003 Biopharma Revenues, here are the following rankings concerning biopharmaceutical companies:

Top 10 Biopharmaceutical Companies based on 2003 Biopharma revenues

1	Amgen	$7,868
2	Genentech	$2,621
3	Serono	$1,858
4	Biogen Idec	$1,852
5	Genzyme	$1,141
6	Chiron	$1,117
7	MedImmune	$993
8	Gilead	$836
9	Millennium	$244
10	Intermune	$154

Note: In all Top Company profiles, dollar amounts are in millions.

Top 10 R&D Expenditures based on 2003 Biopharma revenues

1	Amgen	$1655
2	Genentech	$722
3	Biogen Idec	$546
4	Serono	$468
5	Millenium	$489
6	Chiron	$410
7	Genzyme	$335
8	Gilead	$165
9	MedImmune	$154
10	Intermune	$120

Note: In all Top Company profiles, dollar amounts are in millions.

Top Royalty Revenues based on 2003 Biopharma revenues

1	Genentech	$500
2	Amgen	$488
3	Chiron	$250
4	Millennium	$190
5	Serono	$161
6	Biogen Idec	$128
7	MedImmune	$62
8	Gilead	$25

Note: In all Top Company profiles, dollar amounts are in millions.

CLASSIFICATION AND ACTIONS OF INFERONS

Inferons or Interferons are a type of glyco-proteins called cytokines that cause an immune response to viral activity in the body and inhibit proliferation of cancerous cells. They were first described in 1957, and were named for their ability to interfere with viruses that are replicating. They are produced in the body by the White Blood Cells (WBC's) and certain types of connective tissue cells called fibroblasts and help regulate the immune system. They can be distinguished from each other by their physical and immunochemical properties and are encoded by natural structural genes. They inhibit viral activity by preventing RNA replication of the invading virus and certain other types of antigens and mark out tumor cells to be destroyed. In human beings, there are three main types of interferons (IFNs) namely: type I, type II and type III. Type 1 interferons consist of 13 types of Alpha (α) (leukocyte interferon), a single type of Beta (β) (fibroblast interferon), Omega (ω), Epsilon (ε) and Kappa (κ) isoforms. Type I IFN's are generally found in mammals and work by binding to a characteristic type of cell receptor known as IFNAR. Type II interferons consist solely of IFN Gamma (γ) (immune interferon). IFN γ binds to a specific type of receptor known as IFNG receptor. Type III interferons were recently discovered and consist of IFN Lambda (λ) and its isoforms which bind specifically to a receptor complex consisting of the receptors IL (Interleukin) 10R2 and IFNLR1. In addition to the Type I interferons already discussed; IFN Zeta (ζ) in mice, IFN Nu (ν) in cats, IFN Tau (τ) in ruminants and IFN Delta (δ) in pigs have also been identified.

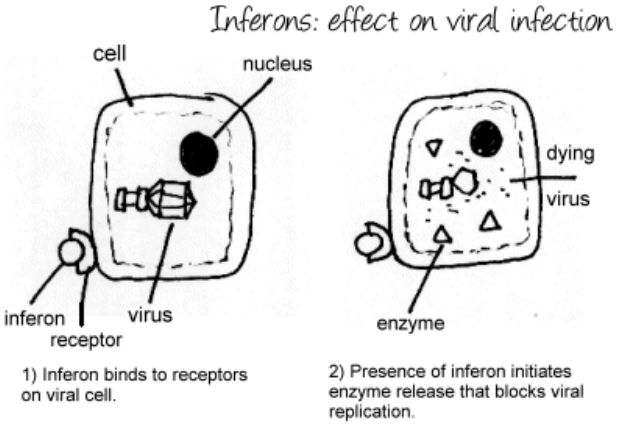


Figure 1: Effects of interferons on invading antigens.
Courtesy *http://stephs-bandanas.celesig.co.uk/guides/chemotherapy_printable.html*

Type I interferons are produced in response mainly by macrophages and neutrophils in response to antigen products such as viral glycoproteins, viral RNA, bacterial endotoxin, flagella, CpG DNA etc. Their metabolism and excretion take place mainly in the kidneys. Their binding to specific receptors on the target antigen cells results in the production of over thirty different kinds of proteins which alerts the T and B cells constituting the WBC's, alerting them of their presence. The T- cells then attack and lyse the invading antigens. They also inhibit viral replication through the enzymes - RNA dependant protein kinase (PKR) and 2-5A synthase which degrade the mRNA and inhibit protein synthesis needed for replication. Figure 1 provides a rough Illustration of the action of interferons on invading bacteria, parasites, viruses etc. Inferons are also active against tumors by marking out them for cytotoxin T-cells, which attack and lyse them. This is illustrated in Figure 2. Once a T-lymphocyte attacks a certain cell it produces antibodies, which will then attack any similar cells. Interferon γ is involved in regulation of inflammatory responses. It is produced by activated T-cells. It generally has weak effects towards viral and cancerous agents but potentiates the effects of interferon α and β. Interferon γ is released by

Th1 cells and recruits leukocytes (WBC's) to the site of the infection resulting in increased inflammation. It also stimulates macrophages to kill engulfed bacteria within the body. It is related to immuno-regulation and has been implicated in auto-immune disorders. Interferons are also involved in the alteration of cell membranes and cytoskeletons, stimulation of cell differentiation, modulation of growth factor expression and reversal of malignant phenotypes. Hormone-like activities of interferons are observed in the cells of the central nervous and neuro-endocrine system. Interferons are also known to modulate central opiod functions, induce alteration is sleep and behavior patterns when administered systemically. IFN-α significantly increases neural activity in the Hippocampus and Somatosensory cortex.

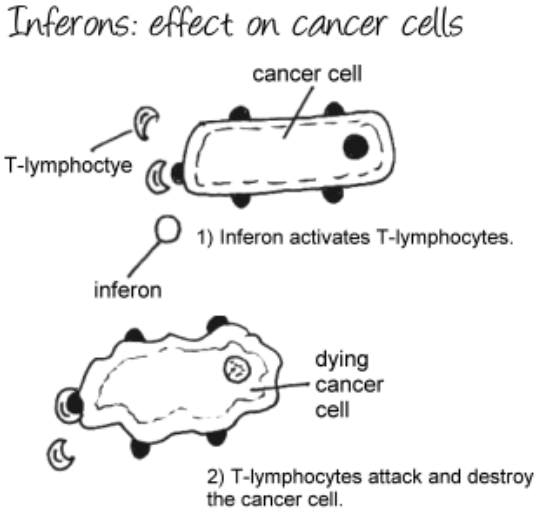


Figure 2: Effects of interferons on tumor cells. Courtesy *http://stephs-bandanas.celesig.co.uk/guides/chemotherapy_printable.html*

Relevant to our field of study is Interferon alpha (IFN – α) which is produced by monocytes and macrophages, lymphobastoid cells, fibroblasts and a number of different cell types following a viral invasion. Murine IFN-alpha variants 4 and 6 can be produced through CHO (Chinese Hamster Ovary) cell lines. At least 23 different variants of IFN-alpha are known. The individual proteins have molecular masses between 19-26 kDa and consist of proteins with lengths of 156-166 and 172 amino acids. All IFN-alpha subtypes or isoforms possess a common conserved sequence region between amino acid positions 115-151 while the amino-terminal ends are variable. Naturally occurring variants also include proteins truncated by 10 amino acids at the carboxy-terminal end.

INTRODUCTION TO IFN-ALPHA

Interferons (IFNs) appear early after viral infection locally and systematically to limit spread of viral infection through antiviral, antiproliferative, or immunomodulatory biologic effects. Upon viral infection, interferons are released into the bloodstream or intracellular fluid to induce healthy cells to manufacture an enzyme that counters the infection. They also affect cell differentiation, growth, surface antigen expression and immunoregulation. There are three naturally occurring interferons: alpha, beta and gamma. Interferon alpha (IFN-alpha, IFNa) is also known as leukocyte interferon. They have a length of 1-2 kb and are clustered on human chromosome 9p22. B-lymphocytes are the predominant cellular producers of INF-alpha. IFN-alpha is derived from lymphoblastic tissue. IFN-alpha comprises a family of related, homologous proteins, each exhibiting a unique activity profile. The activities of the different IFN-alpha species on viruses can vary twenty fold or more. It is not known whether all these genes are actually expressed following simulation of these cells and in some cell systems expression of some subtypes (IFN-alpha-1, IFN-alpha-2, IFN-alpha-4) is stronger than those of others.

INF-alpha has a number of therapeutic applications in the treatment of various human cancers and diseases of viral origin. Recombinant IFN-alphas from both natural and synthetic genes bind to a common cell surface receptor and induce anti-viral activity in a variety of cell lines. IFN-alpha has emerged as an important regulator of growth and differentiation, affecting cellular communication and immunologic control. The efficacy of IFN-alpha has been shown in many different diseases of viral, malignant, angiogenic, allergic, inflammatory, and fibrotic origin. Recent data suggests that IFN-alpha is a multifunctional immunomodulatory cytokine with profound effects on the cytokine cascade including several anti-inflammatory properties. These newly identified immunoregulatory and anti-inflammatory functions may be of importance in the treatment of diseases such as chronic viral hepatitis and help explain some of the interferon mechanisms.

IFN-alpha is mainly used for the treatment of hairy cell leukemia (HCL), metastasizing renal carcinoma, malignant melanoma, and AIDS-related angiogenic tumors. The treatment against cancer is one of the major applications of IFN-alpha. The body recognizes foreign, diseased, or cancerous cells by special marks on their surfaces called antigens. These marks allow the body's immune system to distinguish them from healthy cells in your body. When this occurs, the immune response sends fighter cells to destroy invading cells. Cells of the body that have been stimulated will begin to produce interferons and other natural substances. These substances not only combat foreign invaders, which may cause infection, they can also prevent the growth and spread of other diseased cells.

Some of the commercial versions of synthesized IFN-alpha are IFN-alpha (2a) also known as Roferon-A, IFN-alpha (2b) (Intron A), IFN-alpha (n3) (Alferon N) and IFN-alpha (con-1) (Infergen). IFN- α 2a is a highly purified protein which consists of 165 amino acids and can be obtained also from the fermentation of the bacteria Escherichia Coli (E-Coli). It is indicated for chronic phase philadelphia (Ph), chromosome-positive chronic myelogenous leukemia (CML) and chronic hepatitis C. IFN- α 2b differs from IFN- α 2a by only one amino acid at position 23 where it has an arginine instead of lysine residue. It is indicated for condylomata acuminata (CA), chronic hepatitis B, malignant melanoma and follicular non-Hodgkin's lymphoma. Both Roferon-A and Intron-A are administered through intravenous or subcutaneous injections. IFN- α n3 can be obtained from human leukocytes induced by a murine virus. It is used only in the treatment of venereal or genital warts and is currently approved only for intralesional use. Infergen contains a 166 amino acid sequence which 30% identity with IFN-beta and 60% sequence identity with IFN-gamma. It is indicated for the treatment of chronic hepatitis C viral infections. Some of the adverse effects of using these products include flu-like symptoms, decreased appetite, weight loss, insomnia, hair loss, mood changes, decreased production of leukocytes and platelets and impaired memory.

IFN-alpha remains the most frequently used IFN for both research and clinical applications. Anti-viral applications such as chronic Hepatitis B and C now make up the bulk of

IFN sales. Different countries throughout the world have approved Hu-IFNa for different applications include: Chronic Hepatitis B, Chronic Hepatitis C, Hairy Cell Leukemia, Kaposi's Sarcoma (AIDS-related), Cutaneous T-cell Leukemia, Chronic Myeloid Leukemia, Renal Cell Carcinoma, Non-Hodgkin's Lymphoma, Adjuvant Therapy for Malignant Melanoma, Bladder Cell Carcinoma, Colon Carcinoma, Laryngeal Papillomatosis, Cervical Dysplasia, Condylomata Acuminata (Venereal Warts), Multiple Myeloma.

IFN-alpha was approved by the Federal and Drug Administration (FDA) on February 25, 1991 to treat hepatitis C and is the first effective treatment against this form of hepatitis, which affects and estimated 150,000 Americans each year.

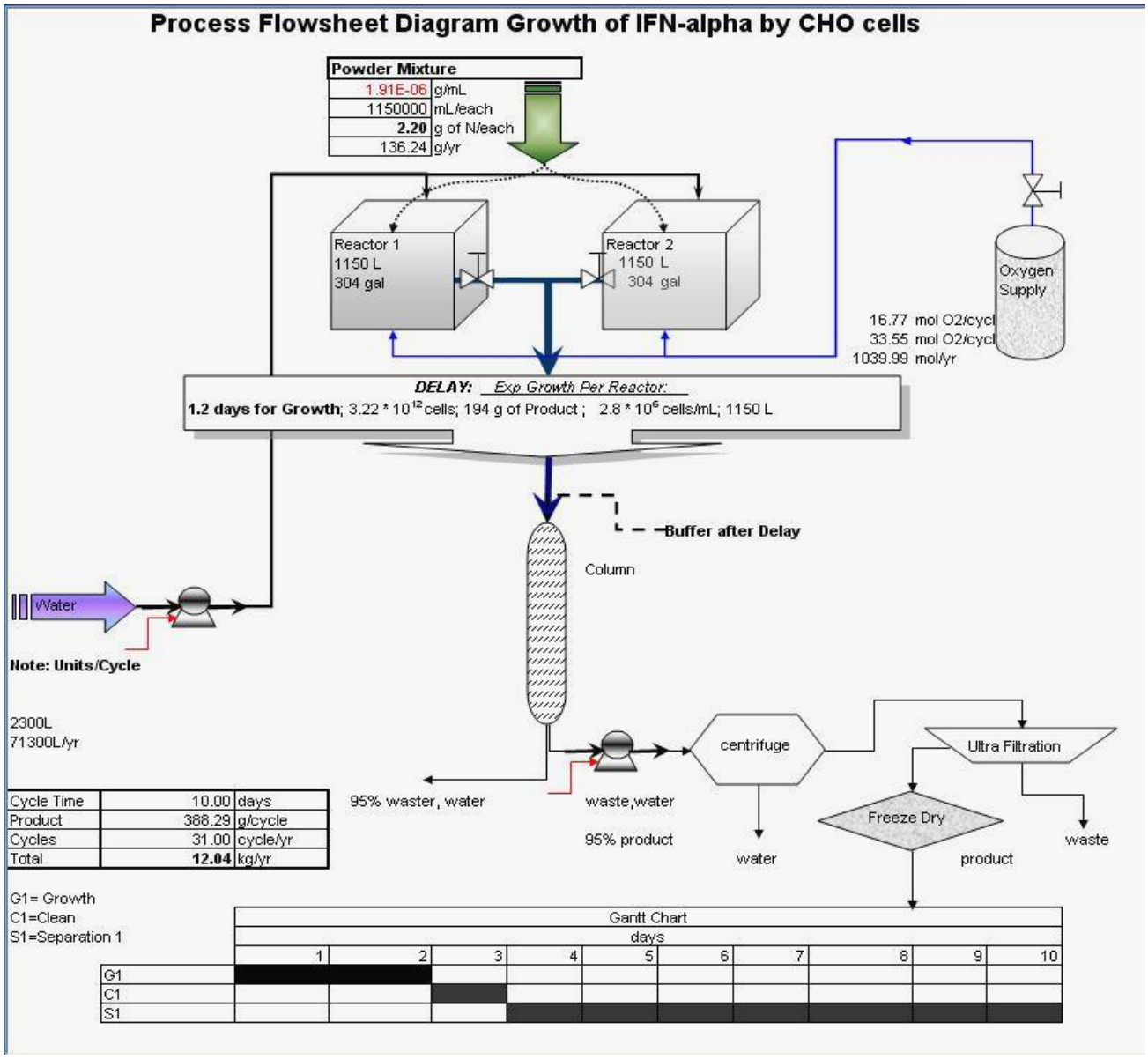
Typically, about half of patients treated with interferon respond with better blood tests and better liver biopsies and approximately half of the patients who respond relapse once the interferon is stopped. IFN-alpha works more effectively the sooner it is used upon the onset of infection, but the symptoms of hepatitis C have been shown to worsen upon end of treatment. In one study, half of the chronic hepatitis C sufferers who had responded to IFN-alpha had a response within six months after the treatment was stopped. The current treatment for hepatitis C involves injections of IFN-alpha three times a week for six months and this usually ranges in cost of about $75 a week. Common side effects of this treatment include flu like symptoms, a reduction in $CD8^+$ T-cells, and a decrease in platelet population. Factors most commonly associated with response to IFN-alpha include: (1) absence of fibrosis or cirrhosis in the pretreatment liver biopsy, (2) lower RNA levels in the blood, and (3) a shorter duration of infection. Essentially, there is no cure against chronic Hepatitis C and progression into cirrhosis or a liver-carcinoma is almost inevitable, but IFN-alpha therapy has been shown to effectively provide about at least a 20% chance that the virus will be killed and aids in the stabilization of the liver.

COMPANIES INVOLVED IN PRODUCTION OF IFN-α

The systemic drug IFN-α is commercially marketed under the brand names Alferon, Roferon, Intron and Wellferon in the countries of the United States and Canada. It is used as an antineoplastic and biological response modifier. They are synthetically produced to replace naturally produced interferons by cells in the body to help fight infections and tumors. Alpha interferons are used to treat hairy cell leukemia, malignant melanoma, and AIDS-related Kaposi's sarcoma. The following table lists the names of major companies that engage in the production of IFN-α, among other products. We should point out that due to the fact that most of these biotherapeutical companies are private, for several of them we have not been able to obtain information regarding the sales and/or income corresponding to the year 2005. For those we did, all information has been found in their respective web pages, or made available directly by a customer service representative.

COMPANY NAME	WEBSITE	2005 SALES (MIL.)	2005 NET INCOME (MIL.)
Hoffmann-La Roche Inc.	http://www.rocheusa.com	N/A	N/A
Biogen Idec Inc.	http://www.idecpharm.com	$2,211.6	$25.1
GTC Biotherapeutics, Inc.	http://www.transgenics.com/	$ 4.2	$30.1
NaPro BioTherapeutics (Tapestry Pharmaceuticals)	http://www.tapestrypharma.com	N/A	$17.6
Promega	http://www.promega.com/default.asp	$169.5	N/A
Anogen	http://www.anogen.ca/	N/A	N/A
Antigenix America Inc.	http://www.antigenix.com/	N/A	N/A
PBL Biomedical Laboratories	http://www.interferonsource.com/	N/A	N/A
Endogen	http://www.endogen.com	N/A	N/A
Biomeda Corporation	http://biomeda.com/?s=1143665908-960962911	N/A	N/A
American Research Products	http://www.arp1.com/	N/A	N/A
Bender MedSystems	http://www.bendermedsystems.com/	N/A	N/A

PROCESS DESIGN



PROCESS DESCRIPTION

CELL PREPARATION:

Upon viral infection, interferons are released into the bloodstream or intracellular fluid to induce healthy cells to manufacture an enzyme that counters the infection. IFN-alpha is normally expressed by B lymphocytes, but has expression levels in all lymphocytes. To stimulate secretion of IFN-alpha from cells, an activator such as endotoxin lipopolysacharide (LPS), dendritic cells, or another more specific activator will be utilized to activate secretion from a sample of clinically acquired peripheral-blood derived lymphocytes (PBL). After approximately 12-18 hours of stimulation, a population of lymphocytes will be collected and will be appropriately lysed and RNA will be isolated from the cells through the basic steps of RNA isolation: (1) cellular lysis and membrane disruption, (2) inhibition of ribonuclease activity, (3) deproteinization, and (4) recovery of intact RNA. RNA isolation is done primarily through the effects of DNAase and the reagent guanidinium isothiocyanate, but the presence of RNAse inhibitor allows for the recovery of the maximum efficiency of intact RNA.

Upon recovery of RNA, the technique of Real Time RT-PCR will allow for the simultaneous conversion of mRNA to cDNA using the techniques of RT-PCR, the PCR amplification of the specific DNA sequence by adding IFN-alpha gene-specific primers with restriction enzyme sites encoded on each end, and the quantification of RNA through absolute quantification and relative quantification to the housekeeping gene. Once the appropriate DNA fragment containing human IFN-alpha is obtained, the insert can be amplified using polymerase chain reaction (PCR) and then ligated into a cloning vector of choice using using basic molecular biology techniques of restriction enzyme digestion, PCR amplification, alkaline lysis, etc. Once the appropriate insert fragment is constructed, it can be ligated (using DNA ligase) into the cloning vector of choice that contains (1) promoters for both bacterial and viral expression and (2) selection markers for both bacterial and viral cell cultures. A cloning vector that seems most suitable is pCDNA 3.1/C7-GFP-TOPO (6157 bp). Insertion of the IFN-alpha DNA fragment into a cloning vector will provide means of verifying the correct amplification and acquisition of the IFN-alpha gene.

The incorporation of the IFN-alpha gene into the cloning vector of choice allows for a stable expression of IFN-alpha, but this cloning vector does not offer means of separating IFN-alpha once it is produced in the presence of Chinese Hamster Ovary (CHO) cells. In this particular situation whereby quantities of IFN-alpha are hoped to be isolated upon mass production using a batch reactor, the IFN-alpha secretion will be separated using the established methodology of His-tagged protein purification. Histidine-tag, most commonly known as "His-tag," is the most commonly used tag for the facilitation of the purification of expressed recombinant proteins. The genetic engineering of tagged recombinant proteins presents a range of advantages. Tags can facilitate the detection of recombinant proteins during expression, but most importantly, tags allow simple one-step purification by affinity chromatography resulting in high purity. Tags can also give additional advantages, such as increasing protein stability and solubility as well as allowing on-column refolding.

Tagged protein purification media and prepacked columns are developed for rapid, one-step purification of unclarified cell lysates (HisTrap FF crude columns) as well as pretreated cell lysates and cell-free systems. The media and prepacked columns permit manual purification with syringes through to automated purification of both unclarified and clarified crude cell lysates as well as on systems, such as ÄKTAdesign, to give high purity and yield.

Purification of tagged proteins is typically based on specific interactions between the tags and ligands. The two most commonly used tags are glutathione S-transferase (GST*) and polyhistidines (His*). Histidine-tagged proteins have a high selective affinity for Ni^{2+} and a variety of other immobilized metal ions. Consequently a protein containing a histidine-tag is selectively bound to a metal-ion charged medium while other cellular proteins bind weakly or are washed out with the binding or wash buffers. Histidine-tags are small and therefore less disruptive to the

properties of the proteins on which they are attached. Consequently tag removal may not always be a priority.

For high-level, secreted expression of IFN-alpha and its application in His-tagged protein purification, the pSecTag2/Hygro designed by Invitrogen® is the particular vector of interest (Figure 3). It is a mammalian expression vector designed for the secretion, purification, and detection of fusion proteins. Each vector has a large multiple cloning site in three reading frames to simplify cloning in frame with the N-terminal secretion signal. The vectors offer the following features: (1) secretion signal from the V-J2-C region of the mouse Ig kappa-chain for efficient secretion of recombinant proteins, (2) cytomegalovirus (CMV) promoter for high-level constitutive expression, (3) C-terminal polyhistidine (6xHis) tag for rapid purification with nickel-chelating resin and detection with an Anti-His(C-term) Antibody, (4) C-terminal c-*myc* epitope for detection with an Anti-*myc* Antibody, (5) Bovine Growth Hormone (BGH) polyadenylation signal and transcription termination sequence to enhance mRNA stability, (6) SV40 origin for episomal replication and simple vector rescue in cell lines expressing the large T antigen (*e.g.,* COS-1, COS-7), and (7) the Hygromycin B resistance gene for selection of stable mammalian cell lines.

Thus, the IFN-alpha gene will be appropriately excised from the pCDNA 3.1/C7-GFP-TOPO vector and ligated into the pSecTag2/Hygro vector using the established methodology of molecular biology cloning techniques. Upon successful ligation into this vector, the IFN-alpha/pSecTag2/Hygro vector can be appropriately transfected into CHO cells to allow for the secretion of IFN-alpha. A stable transfection will first be developed to ensure a consistent and optimized secretion level of IFN-alpha. After acquiring a stable transfection, the IFN-alpha/pSecTag2/Hygro transfection into CHO cells will be placed in the batch reactor system and will be consequently be scaled up for higher production levels of IFN-alpha.

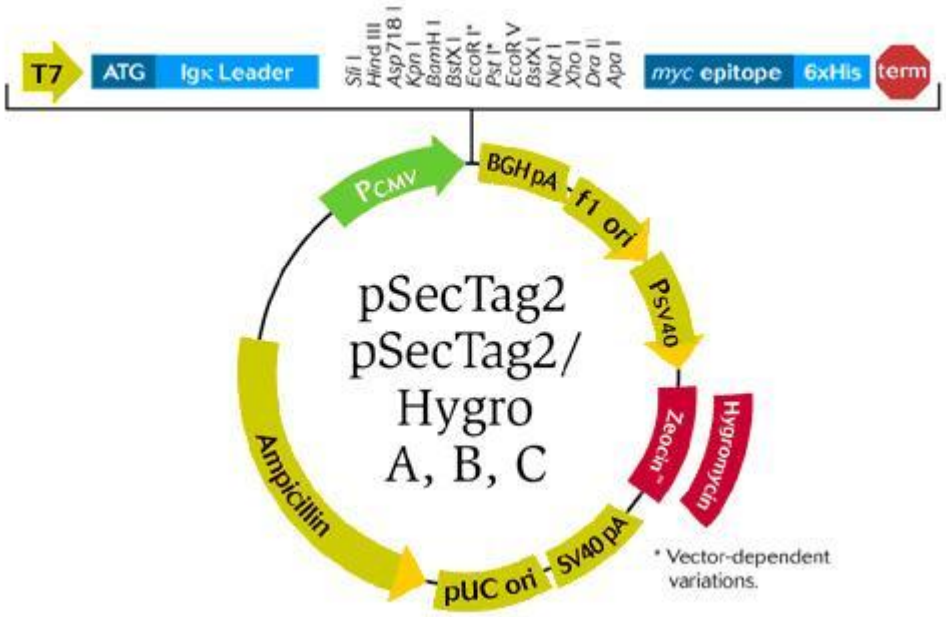


Figure 3: Vector pSecTag2 and pSecTag2/Hygro are both vectors that enable high-level secreted expression of a histidine-tagged protein for purification using a nickel-affinity column

BATCH REACTOR

This is where the Chinese hamster cells grow and secrete the product (IFN-alpha). The cells are put into the batch reactor where it grows on a nutrient. The nutrient media is HYQ-PF-CHO. This is a mixture of nutrients, salts, amino acids, insulin, and transferring specifically designed for the growth of CHO cells. The media comes in form of powder which is dissolved in water. The cells in the batch reactor grow on this mixture.

The fermentation reaction occurs at aerobic conditions with oxygen consumption of $0.18\times10^{-12} mol/cell.hr$ at 30 ^{o} C and 1 atm.

We were given the following constants for the batch reactor calculations:

$\mu\max = 0.5\times10^{6} day^{-1}$ $K_s = 40 gm/L$

Maximum cell concentration before degradation = $2.8\times10^{6} cell/ml$

Biomass Yield = $50\times10^{-12} gm/cell.day$

The cell growth rate in a batch reactor can be expresses by the following differential equation:

$$\frac{dC_c}{dt} = r_g - r_d$$

Where r_g =cell growth rate, $cells/ml.day$, r_d =cell death rate, $cells/ml.day$, C_c =cell concentration, $cells/ml$. Since we are given a maximum cell concentration before degradation, we do not let the cells reach their death rate. So we can equate r_d to zero. Therefore,

$$\frac{dC_c}{dt} = r_g \quad r_g = \mu C_c \text{ so } \frac{dC_c}{dt} = \mu C_c$$

Solving the differential equation:

$$\int_{C_{c0}}^{C_c} \frac{dC_c}{C_c} = \int_0^t \mu dt \qquad \left[\ln C_c\right]_{C_{c0}}^{C_c} = \left[\mu t\right]_0^t \qquad \ln\left(C_c / C_{c0}\right) = \mu t$$

$$\left(C_c / C_{c0}\right) = \exp(\mu t)$$

$$C_c = C_{c0}\exp(\mu t)$$

Where C_{c0} =Initial cell concentration in $cells/ml$, t = reaction time, days and μ = specific growth rate in day^{-1}. The specific cell growth rate can be expressed as $\mu = \mu\max\left(\frac{C_s}{C_s + K_s}\right)$

Therefore, $C_c = C_{c0}\exp(\mu\max\left(\frac{C_s}{C_s + K_s}\right)t)$

Using this equation we can calculate the final concentration has a function of initial concentration, substrate concentration and the reaction time.

We have values for $\mu\max$ and K_s, so the parameters we work with are the initial cell concentration (C_{c0}), substrate concentration (C_s) and the reaction time (t).

We have two batch reactors, each 1150 Liter large connected in parallel. They function at the same time.

One CHO cell is charged into each reactor, so the initial cell concentration is:

$$C_{c0} = \frac{1cell}{1150L} = \frac{1cell}{1150000ml} = 8.7\times10^{-7}\ \text{cell/ml}$$

With a substrate concentration $(C_s) = 1.91\times10^{-6} gm/ml$

It takes 1.2059 days to reach the maximum cell concentration before degradation ($2.8\times10^{6}\,cell/ml$).

Since we reach the maximum concentration, we can calculate the amount of cells produced for each reactor.

Amounts of cell produced = $C_c \times V = \frac{2.8\times10^{6}\,cells}{ml}\times1150000ml = 3.22\times10^{12}\,cells$

Based on the biomass yield given, the reaction time and the amount of cells produced, the amount of product (IFN-alpha) from each reactor can be calculated.

Protein (IFN-alpha) = $3.22\times10^{12}\,cells\times\frac{50\times10^{-12}\,gm}{cell.days}\times1.2059\text{days} = 194.1441761\text{gm}$

Since we are operating two reactors, the total amount of protein produced per cycle:
Protein produced per cycle= $194.1441761\times2 = 388.2883522\text{gm/cycle}$

Since cell preparation takes 21 days and leaving 34 days of the year for maintenance.

Days left for cycles a year = $365-(21+34) = 310days/yr$

We have 310 days in the year to carry out a certain amount of cycles.

We have approximately 2 days to carry out the batch reaction, 1 day for cleaning the reactor after the batch reactor and 7 days for the separation of the protein from the cells, nutrients and waste. So altogether, it takes 10 days for a cycle to be complete. Therefore

Number of cycles a year= $\frac{310days}{yr}\times\frac{1cycle}{10days} = 31cycles/yr$

Therefore the amount of IFN-alpha produced a year is

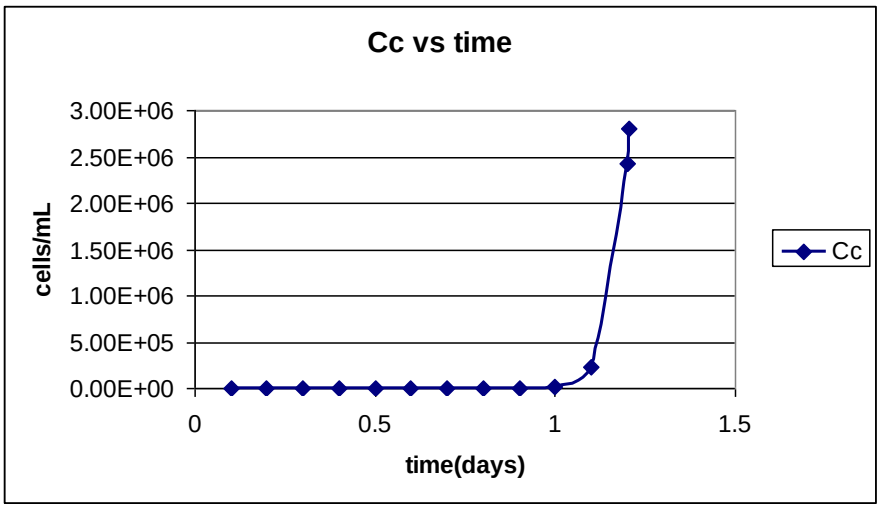
IFN-alpha/yr= $\frac{388.2883522gm}{cycle}\times\frac{31cycles}{yr} = 12035.89848\text{gm/yr} \cong 12\text{kg/yr}$

This is slightly above our process capacity of 10kg/yr. But this is done for a reason. During the recovery process, a considerable amount of IFN-alpha is lost in each unit-operation. So going above the process capacity is just to compensate the amount of product lost during the separation process. The final amount comes to 10kg/yr.

Days	Cc
0.1	9.47E-06
0.2	1.03E-04
0.3	1.12E-03
0.4	1.23E-02
0.5	1.34E-01
0.6	1.45E+00
0.7	1.59E+01
0.8	1.73E+02

0.9	1.88E+03
1	2.05E+04
1.1	2.23E+05
1.2	2.43E+06
1.205864	2.80E+06

Red indicates that the maximum cell concentration is exceeded after approximately 1.2 days



SEPERATION:

NICKEL AFFINITY COLUMN:

The batch reactor system will allow for the production of IFN-alpha-Histag from the CHO cells. The secretion of Histidine-tagged IFN-alpha allows for isolation and purification of only IFN-alpha from the products of the batch reactor systems. The Ni Sepharose™ 6 Fast Flow resin available from GE Healthcare Life Sciences ® is optimized for optimized for high-performance purification of histidine-tagged* proteins. It has a negligible leakage of Ni^{2+} ions, and has a high binding capacity of at least 40 mg/ml medium. Essentially, this is a gravity flow purification system which allows for separation of histidine-tagged proteins. The precise protocol for optimal separation will need to be developed in detail after a few initial experimental trials, but the basic function of this nickel-affinity histidine-tagged protein purification system is that histidine-tagged proteins will be bound to the resin based on their Ni^{2+} affinity once a binding buffer is passed through the column and then an elution buffer can be used to separate only the successfully histidine-tagged IFN-alpha proteins. The binding buffer that will be utilized is recommended to be 20 mM sodium phosphate, 0.5 M NaCl, 20-40 mM imadazole, pH 7.4 and the recommended elution buffer is 20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4. In particular, the concentration of imidazole that will give optimal purification results in terms of purity and yield is protein-dependent and will need to be experimentally derived.

The dynamic binding capacity of this protein purification system is 40 mg/mlL and the Ni Sepharose 6 Fast Flow can be run several times while maintaining the same capacity. The precise number of times that the resin can be reused without replacing it with a fresh stock is purely dependent on the nature of the sample, but the nature of the binding capacity is largely based on the amount of Ni^{2+} in the eluent. This will be periodically quantified using a quantitative measurement assay in which the medium will be run with 10 column volumes of pH 4.0 buffer and the Ni^{2+} still bound in the column will be measured spectroscopically and compared with the amount of Ni^{2+} initially loaded. In the event that the amount of Ni^{2+} decreases in the column, the

Ni Sepharose 6 Fast Flow can be stripped and regenerated with 50 mM EDTA in 20 mM sodium phosphate, 0.5 M NaCl, pH 7.4, and recharged with Ni^{2+}.

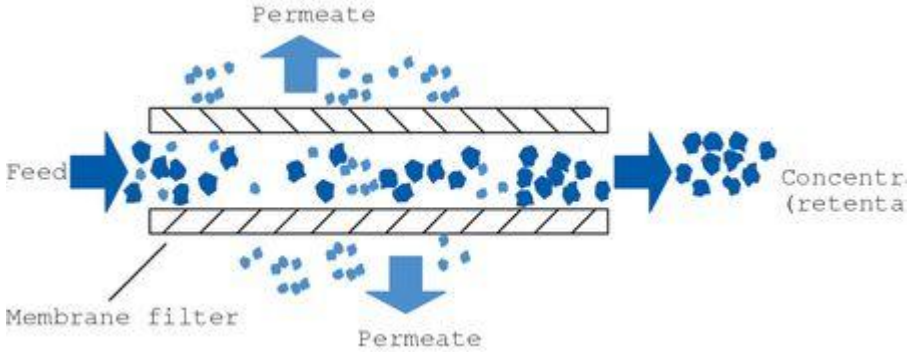
Upon final elution with the recommended elution buffer, the elution will contain Histidine-tagged IFN-alpha protein secretion bound to imidazole. The presence of the Histag fusion to IFN-alpha will not affect the function of the IFN-alpha protein and, thus, does not need to be removed, but the imidazole needs to be removed from the target protein to minimize any impairment to the functionality of IFN-alpha. Imidazole can be removed with the appropriate procedures recommended by GE Healthcare Life Sciences ®.

CENTRIFUGE:

After obtaining the final elute of Histidine-tagged IFN-alpha, the elution will be centrifuged to separate any remaining impurities in the elution. This is only the initial step in the final purification of the eluted IFN-alpha protein.

ULTRAFILTRATION:

Ultrafiltration (UF) is a variety of membrane filtration in which hydrostatic pressure forces a liquid against a semipermeable membrane and consequently seperates species by molecular size or shape. Suspended solids and solutes of high molecular weight are retained, while water and low molecular weight solutes pass through the membrane. This separation process is used in industry and research for purifying and concentrating macromolecular (10^3 - 10^6 Da) solutions, especially protein solutions Essentially, this process will allow the removal of solvent from solution to ultimately result in solute concentration or enrichment. UF membranes may also be used for diafiltration and buffer exchange to remove salts or other microspecies from solution via repeated or continuous dilution and re-concentration. The hollow-fiber UF membranes are anisotropic structures consisting of a thin, dense skin supported by a microporous sub-structure, and characterized using a series of known solutes.



In ultrafiltration, species smaller than the membranes pass across the membrane while larger species are retained.

FREEZE DRYING:

Once the eluted IFN-alpha protein is purified through ultrafiltration, it will then undergo the process of freeze drying to further concentrate the elute and to allow for long-term storage of the protein. Freeze drying not only removes excess water from the elute, but also minimizes the occurrence of protein unfolding or any other protein conformational change, thereby prolonging the functionality of IFN-alpha.

ENERGY BALANCE AND UTILITY NEEDS

There were no need for heaters or coolers in the process because the cell growth occurred at 30°C which is only sligtly above the room temperature of 25°C and there were no exothermic or endothermic processes which would have resulted in a change of cell reactions through the process. The first pump was placed before the batch reactors to ensure that the water was sent in to the batch reactors at the specifed flow rate and the valves were placed after the batch reactors to control the amount of effluent emerging from the reactors. A pump was also placed before the centrifuge to increase the pressure of the effluent from the column.

First Pump (before batch reactors)

Power requirement through pump for incompressible fluid (since main component is water)

$$W = F(h_{out} - h_{in}) = F\int_{P_{in}}^{P_{out}} vdP = Fv\Delta P$$

Volumetric flow rate = 71300 $\frac{L}{hr}$

F = molar flow rate = 71300 $\frac{L}{hr} \times 1\frac{kg}{L} \times \frac{hr}{3600s} \times \frac{1}{18}\frac{kmol}{kg}$ = 1.1 $\frac{kmol}{s}$

v = molar volume = $\frac{2300\ \mathrm{L}}{2300\ Kg} = 1\frac{\mathrm{L}}{kg} = 1\frac{\mathrm{L}}{kg} \times 18\frac{kg}{kmol} = 18\frac{L}{kmol}$

ΔP = (1.5atm-1atm) = 0.5 atm =

$\Delta P = 5.06635\times10^4$ Pa = $5.06635\times10^4 \frac{J}{m^3} \times \frac{1\,kJ}{1000J} \times \frac{1\,m^3}{1000L} = 0.050664\frac{kJ}{L}$

$W = Fv\Delta P = 1.1\ \frac{kmol}{s} \times 18\frac{L}{kmol} \times 0.050664\frac{kJ}{L} = 1.00314\frac{kJ}{s}$

Second Pump (before centrifuge)

Power for incompressible fluid (since main component is mainly water)

$W = Fv\Delta P$

Volumetric flow rate = 231 $\frac{L}{\min}$

F = molar flow rate = 231 $\frac{L}{\min} \times 1\frac{kg}{L} \times \frac{1\min}{60s} \times \frac{1\ kmol}{18\ kg}$ = 0.213889 $\frac{kmol}{s}$

v = molar volume = $\frac{368.6\ \mathrm{L}}{368.6\ Kg} = 1\frac{\mathrm{L}}{kg} = 1\frac{\mathrm{L}}{kg} \times 18\frac{kg}{kmol} = 18\frac{L}{kmol}$

ΔP = (1.7 atm - 1 atm) = 0.7 atm

ΔP = 7.093E$\times10^4$ Pa = $7.093\times10^4 \frac{J}{m^3} \times \frac{1\,kJ}{1000J} \times \frac{1\,m^3}{1000L} = 0.07093\frac{kJ}{L}$

$W = Fv\Delta P = 0.213889\frac{kmol}{s} \times 18\frac{L}{kmol} \times 0.07093\frac{kJ}{L}$ = $0.273081\frac{kJ}{s}$

Valves
Since our valves are well insulated, there is no heat transfer and the enthalpy into the vlave equals that outside so there is no work done or by the valve.

$$W = F(h_{out} - h_{in}) = 0$$

Ultrafiltration
The ultrafiltration step works on the principle of using pressure through a semi-permeable membrane with pores sized to retain solids and pass water like a molecular sieve and so does not need an external power supply to work and so contains no power requirements

Centrifuge
Based on the small amount of material coming into the centrifuge, its power consumption is negligible.

Freeze Drying
Most of the power needed for this stage shall be from the refrigeration stage as seen below

$$W = \left(\frac{T_H - T_C}{T_C} \right) Q_C = \left(\frac{(30 + 273.15) - (5 + 273.15)}{5 + 273.15} \right) 10 = 0.898796 \text{ kJ/s}$$

Where T_H represents the hot temperature in Kelvin and $T_C$represents the cold temperature in Kelvin

$$W = 0.898796 \text{ kJ/s}$$

SAFETY HEALTH AND ENVIRONMENTAL ISSUES

Due to the fact that the plant is being operated at slightly above room temperature conditions, the unit operations do not pose any form of threat to the employees. All the unit operations will be operated at slightly above room temperature conditions aside from the freeze drying unit operation. The purpose of the freeze drying as described above is to solidify and package the IFN-alpha protein. Since no forms of fumes will be emitted, no form of danger will arise from the freeze drying operational units to our employees.

Before the cells go through the batch reactor, it will be necessary to prepare them according to the protocol described in previous sections. The protocol that will be followed in order to express the protein in plasmids is a safe and well studied procedure which has been done many times in the lab without any reports of danger. Also, the cells which are grown inside the batch reactors with the nutrient media, pose no form of threat to our employess on both short term and long term exposure. The resin and buffer which will be used in order to separate the protein are not radioactive and therefore no extra protective equipment would be needed in order to gurantee the safety of our employees. In order to handle the waste of biohazardous material, the plant will dispose of waste according to biohazardous waste regulations.

Before any of our employees begin working in the plant, it will be the responisbility of the plant to train them in all protocols as well as proper disposal methods. Another level of precaution which will be taken by the plant to ensure the safety of our employees would be to make it mandatory that all employees wear protective goggles and protective gloves just for a measure of precaution. If any of the materials gets inhaled, spilled, or ingested by an employee, appropriate MSDS procedures will be follow to insure for the safety of our workers.

Whenever operating a plant, it is always neccesary to think of the worst case scenarios. If it seems that the feed line breaks going into the batch reactor, employees will turn the feed lines off and clean any leaked biohazard material according to specifications. In order to prevent spills from the feed to the batch reactors, a FHA can be installed on the feed line pipes to let workers know when the flow is getting to high so the workers can control the feed flow rate. Also, if any leakage occurs while in the batch reactor, it will be the workers responsibility to clean it up wearing the proper protective gear and follow proper waste disposal procedures as indicated by the Material Safety and Data Sheet.

Besides saftey considerations for our plant and employess, we have also investigated the impact this process will have on the environment. Due to the fact that there exists very little energy requirements during the production of IFN-alpha, it can be said that this process will not be environmentally taxing. No fumes or any forms of chemicals are being released which can be harmful to the people in the plant as well as outside of the plant. All disposal of biohazardous material will comply to proper standards that have been imposed by the government.

DETAILED DESCRIPTION OF INDIVIDIUAL UNIT OPERAIONS

Reactors
The cells and nutrient media will be fed to two parallel batch reactors. The purpose of the batch reactors is to allow the cells to grow and secrete the IFN-alpha. As shown in the Gant chart, two days will be alotted to growth in the batch reactors. After two days in the batch reactor, the cells with the nutrient media and the His-tag bound IFN-alpha will be sent to the nickel affinity column for separation. Once the cells and nutrient media are emptied 1 day will be taken for cleaning of the batch reactors.

Nickel Affinity Column
The purpose of the Nickel Affinity Column will be to separate the His-tagged bound IFN-alpha away from the rest of the nutrient media. Resin will be bound to the nickel affinity column which purpose will be to bind to the His-tagged IFN-alpha and allow separation from the rest of the nutrient media. As shown through the Gant chart, 7 days have been alotted for the separation of IFN-alpha from the nutrient media. In these 7 days is included the time for a buffer to be poured down the column in order to separate the bound protein from the resin, the centrifuge unit operation, the ultrafilration unit operation, and the freeze drying unit operation.

Centrifuge
The purpose of the centrifuge is to separate the buffer from the protein IFN-alpha. On the basis of density, two heterogenous layers will form and the IFN-alpha will be extracted and sent to the the ultrafiltration unit operation.

Ultrafiltration
The purpose of the ultrafilration unit operation is to further purify the IFN-alpha in order to comply with the FDA standards in terms of purity. Once the IFN-alpha has gone through the ultrafilration unit operation it will be sent to get Freeze dried.

Freeze Drying
The purpose of the Freeze Drying is to solidify the IFN-alpha for packaging so it can be shipped to our customers.

COSTS AND OVERALL PROFITABILITY ANALYSIS

COST ESTIMATE

Based on the size and dimensions of our equipments we can estimate the cost of the process plant. For instance, the equation for the estimation of the cost of a centrifuge,

$$C_p = 4300D^{0.94}$$

Where D, is the diameter of the centrifuge in inches:
Our Centrifuge has a diameter of 4.267 inches. Therefore,

$$C_p = 4300(4.267)^{0.94}$$

$$C_p = \$16818.36$$

This is the price to buy a centrifuge of this size; we still have to consider cost of shipping and cost of installation. We multiply the price C_P by the bare module factor F_{BM} to get the overall cost of the equipment C_{FE} .

$$C_{FE} = C_P \times F_{BM}$$

$$C_{FE} = 16818.36 \times 2.03$$

$$C_{FE} = \$34141.27$$

This is the overall cost of our centrifuge; we do the same for our vessels, pumps, ultra filtration, and freeze drying unit. All of these units have different equations for cost estimation, but follows the same principle. Summing all these costs together gives the total bare-module costs for fabricated equipment C_{TFE} .

Equipment	Cp	Fbm	CFE (for equipment)
Pumps	18187	3.3	60017.1
Vessels (Batch Reactors)	10000	4.16	41600
Vessels (Nickel affinity column)	1000	4.3	4300
Centrifuge	16818.36	2.03	34141.27831
Ultra filtration	10000	2.32	23200
Freeze drying	10925.96	2.06	22507.46913
CTFE			185765.8474

We now multiply the total bare-module cost by the cost index, to get the corrected cost.

$$Corrected\,(C_{TFE}) = C_{TFE} \times (\frac{402}{394})$$

$$Corrected\,(C_{TFE}) = \$189537.7$$

Total Bare module investment (C_{TBM}) = $C_{TFE} + C_{PM} + C_{Spare} + C_{Storage} + C_{Catalyst}$

All the proceeding costs are estimated as a percentage of the already calculated C_{TFE} .

Total bare-module costs for fabricated equipment (CTFE)	189537.7
Total bare-module costs for process machinery (CPM)	900
Total bare-module costs for spares (Cspare)	947.6887
Total bare-module costs for storage and surge tanks (Cstorage)	852.9198
Total cost for initial catalyst (Ccatalyst)	125000
Total bare module investment (CTBM)	317238.35

Total direct permanent investment $(C_{DPI}) = C_{TBM} + C_{Site} + C_{Serv} + C_{alloc}$

All these costs are estimated percentages of the already calculated C_{TBM} .

Total bare module investment (CTBM)	317238.35
Cost of site preparation (Csite)	47585.75
Cost of Service Facilities (Cserv)	23792.88
Allocated cost for utility plants and related facilities (Calloc)	27039.26
Total direct permanent investment (CDPI)	415656.2453

Total depreciable capital $(C_{TDC}) = C_{DPI} + C_{cont}$

The contingency cost is estimated as a percentage of the total direct permanent investment.

Total direct permanent investment (CDPI)	415656.2453
Cost of contingencies and contractor's fee (Ccont)	145479.6858
Total depreciable capital (CTDC)	561135.9311

Total permanent Investment $(C_{TPI}) = C_{TDC} + C_{land} + C_{royal} + C_{startup}$

The unknown costs are estimated percentages of already calculated C_{TDC} .

Total depreciable capital (CTDC)	561135.9311
Cost of land (Cland)	11222.72
Cost of royalties (Croyal)	11222.72
Cost of plant startup (Cstartup)	56113.59
Total permanent investment (CTPI)	639694.9615

Total Capital investments $(C_{TCI}) = C_{TPI} + C_{WC}$

Working capital is 8.33% of the annual sales.

Total permanent investment (CTPI)	639694.9615
Working capital (CWC)	41650000
Total capital investment (CTCI)	42289694.96

The total capital investment = $ 42.3 million.

All percentages of estimated cost can be found in Process Design Principles By "Warren D. Seider"

COST SHEET

We prepared an annual cost sheet of our process using estimated cost sheet from Process Design Principles By "Warren D. Seider"

Using Price of IFN-Alpha (1milligram=$50)

Feedstocks	
Utilities	
Steam 450 psig (lb)	0
Steam 150 psig (lb)	0
Steam 50 psig (lb)	0
Electricity (kW-hr)	100000

Cooling Water (gal)	0
Refrigeration (-30F)	100000
Process Water (gal)	18.815
Boiler Water (gal)	0
Chilled Water 40F (ton-day)	0
HYQ-PF	300
CHO-Cells	0
Resin	125000
Buffer	0
Waste Water Treatment (lb)	31402.2
Landfill (lb)	0
	356721.015
Cell Preparation	
Operations	
Direct Wages & Benefits (DW&B)	1248000
Direct Salaries & Benefits (DS&B)	187200
Operating Supplies	74880
Technical Assistance	260000
Control Lab	285000
	2055080
Maintenance	
Wages and Benefits (MW&B) Fluid	19639.75759
Wages and Benefits (MW&B) Solid-fluid	25251.1169
Wages and Benefits (MW&B) Solid	28056.79656
Salaries and Benefits	18236.91776
Materials & Service	72947.67105
Maintenance overhead	3647.383552
	167779.6434
M&O-SW&B	2222859.643
Operating Overhead	
General plant overhead	157823.0347
Mechanical department services	53348.63144
Employee Relations department	131148.719
Business Services	164491.6136
	506811.9987
Property Taxes and Insurance	11222.71862
Depreciation (straight line depreciation)	
Direct plant	42338.36789
Allocated plant	1914.379951
	44252.74784
Cost of Manufacture (COM)	3141868.124

Sales (S)	IFN-alpha (10kg/yr) (1000microgram=$50)	500000000
	General Expenses	
	Selling Expense	5000000
	Direct Research	24000000
	Allocated Research	2500000
	Administrative expense	10000000
	Management incentive compensation	6250000
Total General Expenses (GE)		47750000
Total Production Cost (C)		50891868.12

APPROXIMATE PROFITABILITY ANALYSIS

Using the cost sheet above and our cost estimate for our process, we can perform an approximate profitability analysis to find out how profitable our process is.

Pretax (Gross) Earning = Sales (S) - Total Production Cost (C)
Pretax (Gross) Earning = $500000000 - $50891868.12 = $449108131.9
Pretax (Gross) Earning = $449 million.

With a Combined Federal and state income tax rate of 37%

Net Earnings (Profit) = (1-0.37)× $449108131.9
Net Earnings (Profit) = $282938123.1

Net Earnings (Profit) $\cong$ \$283 million

Return on investment (ROI) = $\frac{Net\_Earnings}{Total\_Capital\_Investment}$

Return on investment (ROI) = $\frac{282938123.1}{42289694.96} = 6.69$

This is a very high return on investment.

Using straight line depreciation, with an annual depreciation of 8%, we can calculate the depreciation.

Depreciation = $0.08 \times Total\_Depreciable\_Capital(C_{TDC})$

Depreciation = $0.08 \times \$561135.9$

Depreciation = $\$44890.87$

We can now calculate the payback period.

$$Payback\_Period = \frac{C_{TDC}}{Net\_Earning + Depreciation}$$

$$Payback\_Period = \frac{\$561135.9}{\$282938123.1 + \$44890.87} = 0.00198 yr$$

This payback is so small, showing that the process is highly profitable. But this is unreasonable because we have a payback period within days of investment.

RIGOROUS PROFITABILITY ANALYSIS

Based on the approximate profitability analysis, we know that the process is profitable. Now we perform a rigorous profitability analysis for a plant life of 15 yrs.

Installation of the plant life takes 3 years. For the first operating year, we operate at 45% capacity, 67.5% capacity for the second operation year and 90% capacity for the third and subsequent years of operation. Also using a more elaborate depreciation scheme, we used the 10 years MACRS depreciation scheme for our process. With an interest rate of 20% and inflation of 4%:

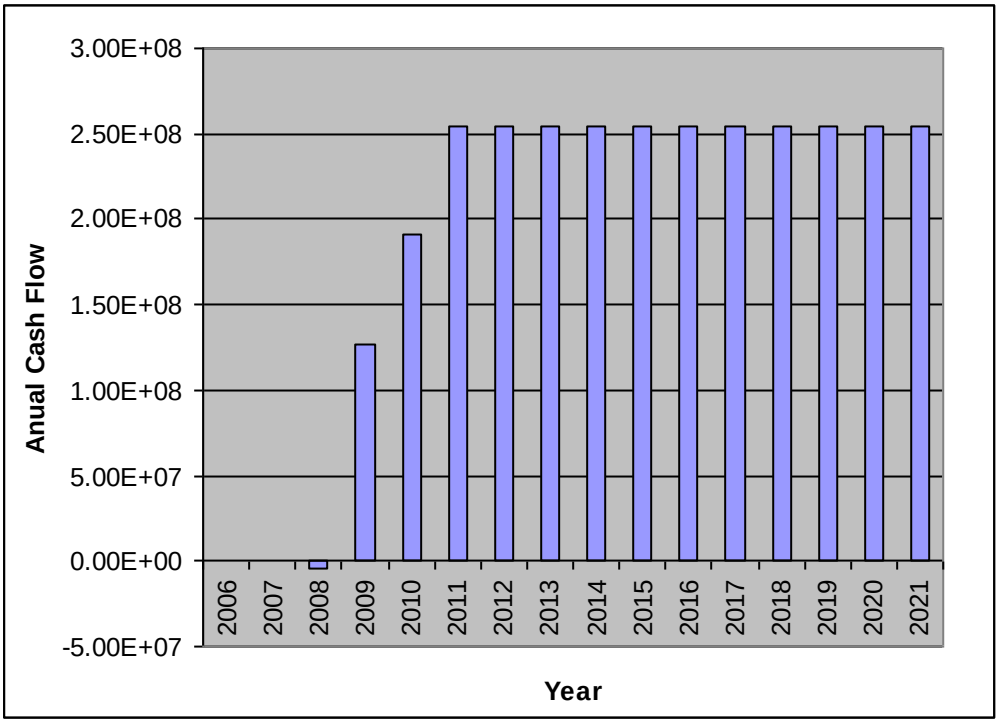
year	Investment	working capital	cost(land, startup, royal)	Cost of sale(excl. Dep)	sales
2006	-187045.31	0	0	0	0
2007	-187045.31	0	0	0	0
2008	-187045.31	-4165000	0	0	0
2009	0	0	-78559.03036	22901340.66	225000000
2010	0	0	0	34352010.98	337500000

2011	0	0	0	45802681.31	450000000
2012	0	0	0	45802681.31	450000000
2013	0	0	0	45802681.31	450000000
2014	0	0	0	45802681.31	450000000
2015	0	0	0	45802681.31	450000000
2016	0	0	0	45802681.31	450000000
2017	0	0	0	45802681.31	450000000
2018	0	0	0	45802681.31	450000000
2019	0	0	0	45802681.31	450000000
2020	0	0	0	45802681.31	450000000
2021	0	0	0	45802681.31	450000000

Dep (%) MACRS	D	Taxable income	Tax Paid
0	0	0	0
0	0	0	0
0	0	0	0
7.37	4135572	202098652	74776501
11.52	6464286	303147977.5	1.12E+08
9.22	5173673	404197309.5	1.5E+08
14.4	8080357	404197304.3	1.5E+08
10	5611359	404197308.7	1.5E+08
18	10100447	404197300.7	1.5E+08
6.55	3675440	404197312.1	1.5E+08
6.55	3675440	404197312.1	1.5E+08
6.56	3681052	404197312.1	1.5E+08
6.55	3675440	404197312.1	1.5E+08
3.28	1840526	404197315.4	1.5E+08
0	0	404197318.7	1.5E+08
0	0	404197318.7	1.5E+08

Net Earn	Cash flow	NPV @ 20%	cum NPV @ 20%
0	-187045.3	-374090.6207	-374090.6207
0	-187045.3	-162105.9357	-536196.5564
0	-4352045	-3027089.294	-3563285.85
127322150.7	131379164	76034474.42	72471188.57
190983225.8	197447512	95219915.71	167691104.3
254644305	259817978	104415029	272106133.2
254644301.7	262724659	87985957.12	360092090.4
254644304.5	260255664	72632579.36	432724669.7
254644299.4	264744746	61571166.55	494295836.3
254644306.6	258319747	50064097.57	544359933.8
254644306.6	258319747	41720081.31	586080015.2
254644306.6	258325358	34767489.65	620847504.8
254644306.6	258319747	28972278.69	649819783.5
254644308.7	256484835	23972067.54	673791851
254644310.8	254644311	19833370.87	693625221.9
254644310.8	254644311	16527809.06	710153031

The graph below shows the discounted annual cash flow over the 15 yr plant life.



We then find the Actual investors rate of return (IRR), by setting the cumulative net profit value of the last year to zero.
The IRR = 588.50%
This further emphasizes how profitable the process is., but this is somewhat not possible because if this were the case, everybody would invest in this process.

NOTE:

This analysis is all based on the price of IFN-alpha (1milligram =$50).
The price of IFN-alpha varies for different companies. It varies from $30 to$250 for 1milligram of IFN-alpha and it depends on the amount of IFN produced by the company. Since we are producing on such a large scale we cannot use the price from companies that produce on a small scale. So we have to go a bit farther with our economic analysis and factor in some humanitarian consideration.

HUMANITARIAN CONSIDERATION

If we were looking too only make high profit, then we would be extremely happy with the price of IFN-alpha and make so much profit. But there are a lot of people that need this product to continue living but due to the high prices cannot afford them. About 150,000 Americans are sick with Hepatitis C, but only a few are fortunate to pay high prices for IFN-alpha which is a successful product for the treatment of this disease. Some companies give out some free product to low income senior citizens; some companies subsidize their product prices to help people overcome these high prices.
So since we are making this product on a large scale, we can suggest a selling price that would be cheap enough for people to buy and we could also profit from.

Now we perform an economic analysis with our new suggested price of:
1milligram = 40 cents.

Feedstocks	
Utilities	
Steam 450 psig (lb)	0
Steam 150 psig (lb)	0
Steam 50 psig (lb)	0
Electricity (kW-hr)	100000
Cooling Water (gal)	0
Refrigeration (-30F)	100000
Process Water (gal)	18.815
Boiler Water (gal)	0
Chilled Water 40F (ton-day)	0
HYQ-PF	300
CHO-Cells	0
Resin	125000
Buffer	0
Waste Water Treatment (lb)	31402.2
Landfill (lb)	0
	356721.015
Cell Preparation	
Operations	
Direct Wages & Benefits (DW&B)	1248000
Direct Salaries & Benefits (DS&B)	187200
Operating Supplies	74880

	Technical Assistance	260000
	Control Lab	285000
		2055080
	Maintenance	
	Wages and Benefits (MW&B) Fluid	19639.75759
	Wages and Benefits (MW&B) Solid-fluid	25251.1169
	Wages and Benefits (MW&B) Solid	28056.79656
	Salaries and Benefits	18236.91776
	Materials & Service	72947.67105
	Maintenance overhead	3647.383552
		167779.6434
	M&O-SW&B	2222859.643
	Operating Overhead	
	General plant overhead	157823.0347
	Mechanical department services	53348.63144
	Employee Relations department	131148.719
	Business Services	164491.6136
		506811.9987
	Property Taxes and Insurance	11222.71862
	Depreciation (staright line depreciation)	
	Direct plant	42338.36789
	Allocated plant	1914.379951
		44252.74784
Cost of Manufacture (COM)		3141868.124
	Price of IFN-alpha (1000microgram = $	0.39854288
Sales (S)	IFN-alpha (10kg/yr)	3985428.803
	General Expenses	
	Selling Expense	39854.28803
	Direct Research	191300.5825
	Allocated Research	19927.14401
	Administrativeexpaense	79708.57605
	Management incentive compensation	49817.86003
Total General Expenses (GE)		380608.4507
Total Production Cost (C)		3522476.574

APPROXIMATE PROFITABILITY ANALYSIS

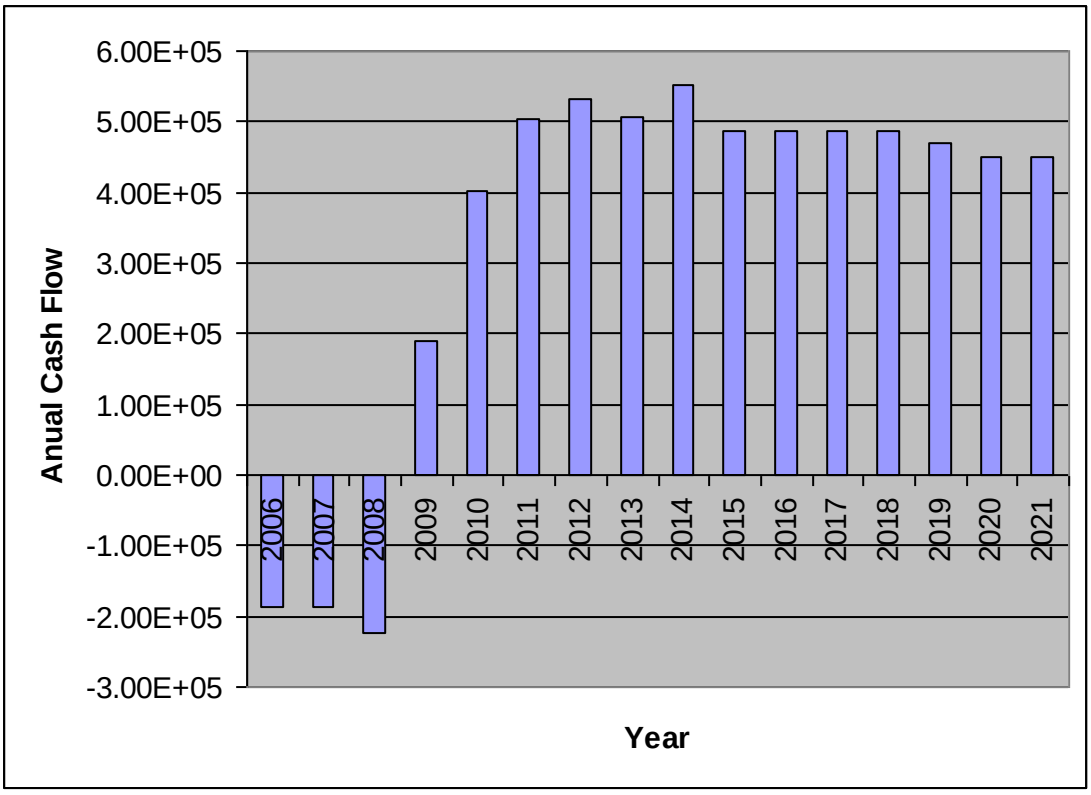
Net profit: $ 291,659
Return on Investment (ROI) = 30%
Payback Period = 1.667 yr

With a Return on Investment of 30% and Payback Period of 1.667 year, the process is still very profitable.

RIGOROUS PROFITABILITY ANALYSIS

Performing the same analysis with the new suggested price gives an Investor's return rate (IRR) of 23.76 %.

Below shows the graph of the discounted annual cash flow:



These shows that even at our suggested subsidized price, our process would still be very profitable.

CONCLUSION AND RECOMMENDATIONS

The process economic analysis proved that the production of IFN-Alpha is a highly profitable process although the initial cost of startup is moderate, the payback period is just over a year and the return on investment is approximately one year. There are minimal safety and environmental issues associated with the plant and economic recommendations for the future include use of a more cost-effective buffer and resin, reduction in the number of separation steps, and possible reduction in the number of batch reactors.

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