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Economic Analysis of Cellulase Production by *Clostridium thermocellum* in Solid State and Submerged Fermentation

Jun Zhuang, Mary A. Marchant, Sue Nokes and Herbert Strobel

Jun Zhuang is a graduate research assistant; Mary A. Marchant is a professor in the Department of Agricultural Economics; Sue Nokes is an associate professor in the Department of Biosystems and Agricultural Engineering; and Herbert Strobel is an associate professor in the Department of Animal Science, University of Kentucky.

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Authors	Contact Information
Jun Zhuang*	Agricultural Economics University of Kentucky 400 C. E. Barnhart Bldg. Lexington, KY 40546-0276 Phone: (859) 323-3394 Fax: (859) 257-7290 Email: junzhuang31@hotmail.com
Mary A. Marchant	(Same as above) E-mail: mmarchan@uky.edu
Sue Nokes	Biosystems and Agricultural Engineering University of Kentucky E-mail: snokes@bae.uky.edu
Herbert Strobel	Animal Sciences University of Kentucky E-mail: strobel@uky.edu

*indicates contact author

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Abstract

Dependence on foreign oil remains a serious issue for the U.S. economy. Additionally, automobile emissions related to petroleum-based, fossil fuel has been cited as one source of environmental problems, such as global warming and reduced air quality. Using agricultural and forest biomass as a source for the biofuel ethanol industry, provides a partial solution by displacing some fossil fuels. However, the use of high cost enzymes as an input is a significant limitation for ethanol production.

Economic analyses of cellulase enzyme production costs using solid state cultivation (SSC) are performed and compared to the traditional submerged fermentation (SmF) method. Results from this study indicate that the unit costs for the cellulase enzyme production are $15.67 per kilogram ($/kg) and $40.36/kg, for the SSC and SmF methods, respectively, while the market price for the cellulase enzyme is $36.00/kg. Profitability analysis and sensitivity analysis also provide positive results.

Since these results indicate that the SSC method is economical, ethanol production costs may be reduced, with the potential to make ethanol a viable supplemental fuel source in light of current political, economic and environmental issues.

Keywords: biomass, enzyme production, ethanol, solid state fermentation, submerged fermentation

Oil consumption by the United States ranks number one, accounting for 25.4% of total global consumption in 2002 (Parry and Darmstadter, 2003). However, with regard to production, the U.S. is the world's third largest oil producer, following Saudi Arabia and Russia, accounting for only 8.6% of global production. In terms of known crude oil reserves, U.S. estimates account for only 2% of global reserves, while the Persian Gulf

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region accounts for two-thirds of reserves (Littell, 2002). The huge gap between U.S. oil consumption and production is filled by foreign oil imports to a large extent, especially from the Middle East, which makes the U.S. vulnerable to potential oil supply disruptions. Not surprisingly, the U.S. Department of Energy, office of Energy Efficiency and Renewable Energy (US-DOE-EERE) has chosen to "dramatically reduce or even end dependence on foreign oil" as their mission statement's first priority (US-DOE-EERE, 2004). Furthermore, according to US-DOE-EERE, automobile emissions related to petroleum-based fossil fuels (e.g., gasoline and diesel) are sources of environmental problems such as global warming and reduced air quality, where large amounts of heat-trapping residue gases are dispersed into the atmosphere when these fuels are incompletely burned (US-DOE-EERE, 2002).

The development of the biofuel ethanol industry provides one partial solution. It is technologically feasible to biologically convert agricultural or forest biomass, such as wheat bran and straw, cornhusks, and rice hulls, into ethanol. This technology is appealing because the raw materials discussed above are inexpensive and available in large amounts in the United States, the world's largest agricultural producer, implying that large amounts of ethanol could be produced to decrease the U.S. dependence on imported oil. Secondly, such technology is inherently a value-added process since valuable biofuels are produced from agricultural wastes. Thirdly, the U.S. Environmental Protection Agency (US-EPA) reported that automobile emissions may be reduced when

ethanol is used as a fuel, compared to conventional gasoline (US-EPA, 2002), which should result in a reduction of global warming and air pollution.

Given the above, adoption of a new technology for large-scale ethanol production from lignocellulose might result in economic and environmental benefits. Unfortunately, a number of factors currently prohibit the commercial production of ethanol from lignocellulose. One main problem is that production costs for enzymes, which is an important facet of the bioconversion process, remains high enough to be a significant proportion of the total costs for ethanol production (Saha and Woodward, 1997). Enzyme production cost estimates range as high as 25 to 50% of the total ethanol production costs (Ruth, 2003; Himmel et al., 1997), which significantly limit the economic viability of this process (Lynd, Wyman and Gerngoss, 1999). While cellulases are traditionally produced by a submerged fermentation (SmF) method, solid state cultivation (SSC) method has the potential to provide cheaper enzymes and therefore may reduce ethanol prices. If economic analysis confirms profitability, ethanol production costs may be reduced, with the potential to make ethanol (from lignocellulose) a viable supplemental fuel source in light of current political, economic and environmental issues.

Although this research focuses on enzyme production in an ethanol context, it is important to note that the availability of low-cost enzymes is significant to other biochemical conversion industries involving biocatalysts. Enzyme production is a growing field of biotechnology with annual world sales close to one billion dollars

(González et al., 2003). The SSC technology discussed in this research would be readily transferable to most bioconversion processes that require enzymes.

The remainder of this paper proceeds as follows: research objectives, literature review, enzyme production simulation, economic analysis and sensitivity analysis, summary and conclusion.

RESEARCH OBJECTIVE

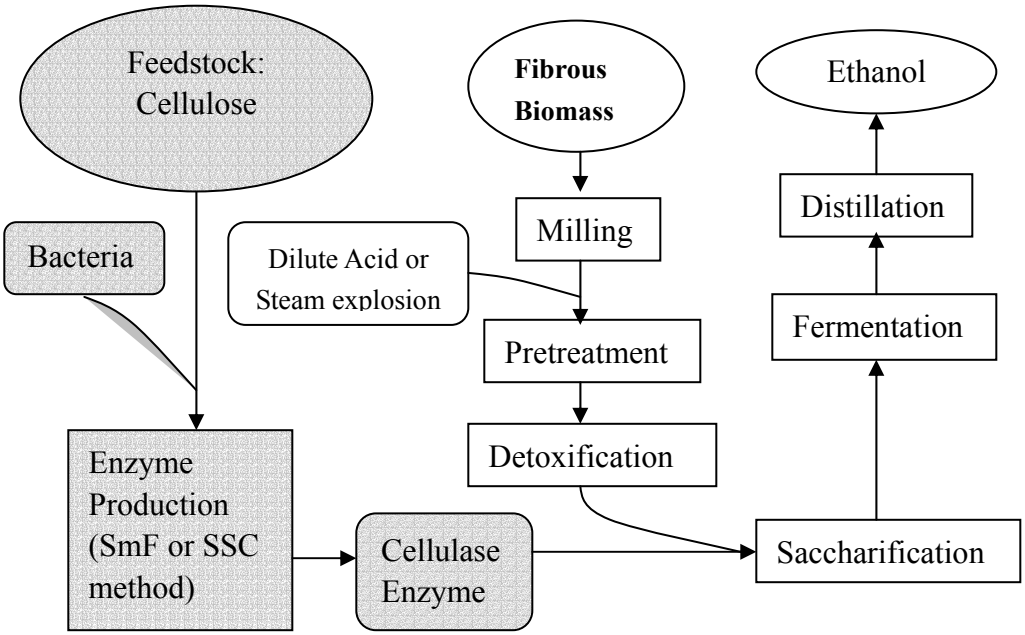
The first objective of this research is to test the hypothesis that the unit costs for cellulase enzyme production using the SSC method is more economical than the traditional SmF method. This objective is realized by conducting unit cost analysis. The second objective of this research is to test the hypothesis that the SSC method is profitable if adopted. This objective is realized by conducting profitability analysis. If the SSC method is economical, ethanol production costs may be reduced, with the potential to make ethanol a viable supplemental fuel source in light of current political, economic and environmental issues.

LITERATURE REVIEW

Enzyme Component in Ethanol Production Process

Enzymes are used as a biocatalyst in ethanol production, specifically in the cellulose saccharification process. Figure 1 represents ethanol production process from fibrous biomass using enzyme saccharification and microbial fermentation.

Figure 1. Enzyme production component within the ethanol production



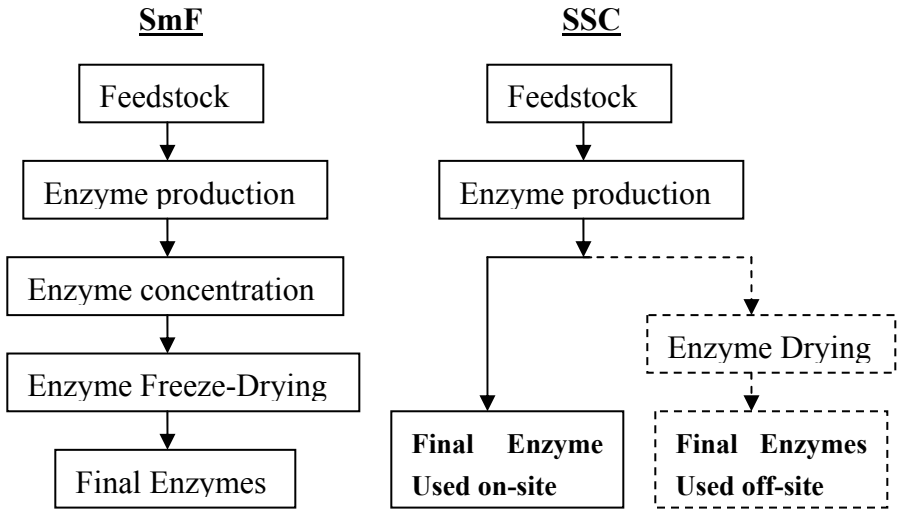
Shaded Area: Enzyme Production Process
Source: Simplified flowchart from Aden et al., 2002.

Enzyme Production using the SmF and SSC Methods

Traditionally, enzymes are produced using the submerged fermentation (SmF) method, in which the cultivation of microorganisms occurs in an aqueous solution containing nutrients. An alternative to the traditional SmF method is the solid state cultivation (SSC) method, which involves the growth of microorganisms on solid materials in the absence of free liquids (Cannel and Young, 1980). Different mediums lead to different downstream processes. The enzymes produced by SmF must be concentrated and freeze-dried before usage because of liquid cultivation. However, the enzymes produced by SSC do not require concentration. It does not have to be

freeze-dried if used on-site. Generally the SSC process is simpler and potentially less expensive than the SmF process. The flow charts are represented in Figure 2.

Figure 2. Flowcharts of enzyme production using the traditional SmF method compared to the SSC Method



(Dashed lines represent off-site enzyme production process)

While SSC is not widely used, it is not a new idea. Foods fermented from moist solids, such as soy sauce and miso soup, have been prepared by SSC for thousands of years in China, Japan, Indonesia and other countries in Asia. However, a glance of history of fermentation technology indicates that the SSC method was nearly completely ignored in Western countries after 1940 due to the adoption of the submerged fermentation (SmF) method (Pandey, 2003). During the last ten years, a renewed interest in SSC has developed due, in part, to the recognition that many microorganisms, including genetically modified organisms (GMO), may produce their products more effectively by SSC (Pandey et al., 1999).

A Comparison between the SmF and SSC Methods

From an economic viewpoint, SSC has at least three advantages over the traditional SmF method for enzyme production: (1) SSC uses much less water and energy than the SmF method. Thus, the SSC method does not require expensive equipment to concentrate or freeze-dry the enzymes, while the SmF method does (also see Figure 2.2). (2) There is almost no effluent from SSC; therefore much less pollution is generated from SSC than SmF. (3) SSC generally results in higher volumetric productivity of enzymes due to a high concentration of feedstock per unit volume within the fermentor. Thus it results in lower unitary capital and operating costs compared to the traditional SmF method (Durand et al., 1997; Kumar and Lonsane, 1987).

Although there are many potential advantages of SSC over the traditional SmF method, there are also some technical problems currently limiting large-scale implementation of SSC. A major problem of SSC is the difficulty in removing the heat generated during microbial growth in a large-scale reactor. This can be more difficult in SSC than in SmF because of the limited heat transfer through the solid substrate (Mitchell, et al., 2003; Deschamps and Huet, 1984). If left uncontrolled, heat accumulation can result in the cessation of mesophilic (moderate-temperature loving) microbial activity therefore the cessation of enzyme production.

To overcome these technical problems, anaerobic, thermophilic (high-temperature) bacteria, *Clostridium thermocellum*, replaces the common aerobic mesophilic (moderate-temperature) bacteria *Trichoderma reesei* in SSC fermentation in this research,

based on our previous laboratory experiments. Heat removal is no longer necessary. No oxygen is required in the culture, and water content control is not an issue in an anaerobic environment. Previous research indicated that *C. thermocellum* can be grown at high temperatures and these technical problems have been overcome. Thus, large-scale enzyme production using the SSC method may become feasible.

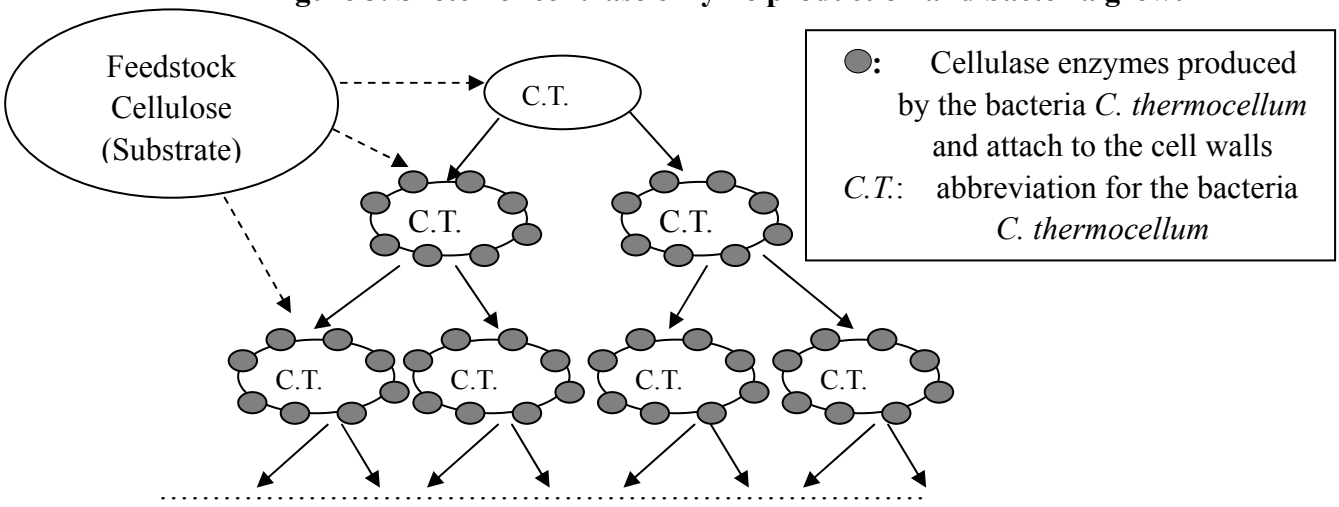
ENZYME PRODUCTION SIMULATION

Enzyme Production Overview

The enzyme production component discussed in this research is a small but costly part of the overall ethanol production process. The process to produce enzymes is fermentation. Since the reactions of fermentations are complex and beyond the scope of this research, the focus of this thesis will center on the growth of the *C. thermocellum* bacteria, which consumes the feedstock cellulose and produce cellulase enzymes (see Figure 3).

Fed with the feedstock cellulose (substrate), the *C. thermocellum* bacteria grows (multiplies) very fast. Cellulase enzymes are produced and attach to the cell walls of the *C. thermocellum* bacteria. A sketch of the growth of the *C. thermocellum* bacteria and corresponding cellulase enzyme production is represented in the Figure 3.

Figure 3. Sketch of cellulase enzyme production and bacteria growth



FLOWSHEETS AND EQUIPMENT OVERVIEW

The traditional SmF enzyme production process requires downstream processes including enzyme concentration and freeze-drying, while the SSC process does not (see Figure 2). Since flowsheets are able to represent the biochemical engineering processes (Peters, Timmerhaus and West, 2003), this section provides flowsheets in Figures 4 and 5 to describe the overall enzyme production processes, followed by a general description of related equipment, for the SmF and SSC processes, respectively.

In the SmF enzyme production process (see the flowsheet in Figure 4), the initial preparation of the bacteria *C. thermocellum* is transferred from a freezer (-80°C) into a sterilized shake flask (SFR-101) containing medium and cellulose. The freezer and sterilizing equipment are assumed economically negligible since their size and therefore costs are small compared with other equipment used in this enzyme production process.

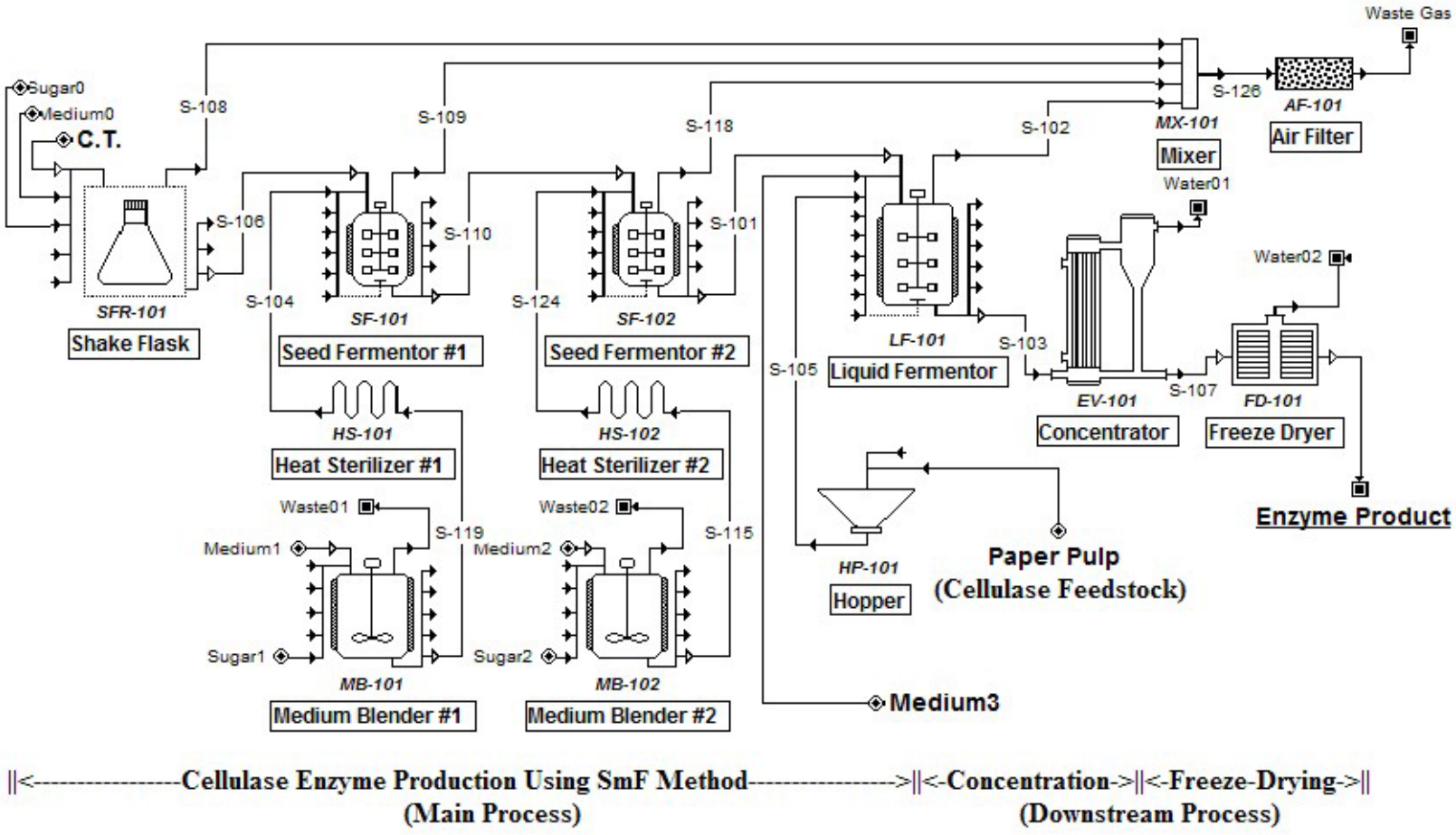
The cultures are fermented in the shake flask (SFR-101) for the first time, transferred to the seed fermentor #1 (SF-101) and fermented for the second time, supplied

by the medium and cellulose (substrate) prepared by medium blender #1(MB-101) and the heat sterilizer #1 (HS-101). Then the cultures are transferred to seed fermentor #2 (SF-102) and fermented for the third time, supplied by the medium and cellulose (substrate) prepared by medium blender #2(MB-102) and heat sterilizer #2 (HS-102). Then the cultures are transferred to the liquid fermentor (LF-101) and fermented for the fourth time, supplied by paper pulp (substrate, containing cellulose) previously stored in a hopper (HP-101). Separate medium is charged into the liquid fermentor.

Nitrogen sweeps are conducted in all vessels --shake flask, fermentors, and medium blenders to guarantee an anaerobic environment. All emission gases from the shake flask and fermentors are emitted into the air through a mixer (MX-101) and an air filter (AF-101). All the other gases are emitted from medium blenders directly into the air.

The product from the liquid fermentor (LF-101) is the cellulase enzyme, together with some residues and water. A concentrator (EV-101) is used to remove water, and the freeze-dryer (FDR-101) is used to further remove water before the contents form the final product--**cellulase enzyme**. The concentration and freeze-drying activities comprise build the downstream process for the SmF method of enzyme production.

Figure 4. The traditional SmF method for producing enzymes –Plant specification



In the SSC process (see flowsheet in Figure 5), this process is largely the same as the SmF process, except for two differences: (1) the paper pulp and medium are sterilized in a sterilizing drum (SD-101), agitated and mixed with the culture transferred from seed fermentor #2 (SF-102) and transferred to the main solid fermentor (SMF-101) using a sterile conveyor (SC-101). The reason that the SSC process requires a sterilizing drum is that stirring is impossible in solid fermentors, while possible for liquid. (2) The final product--**cellulase enzymes**--produced from the solid SSC fermentor is assumed ready to be used on-site, so that there is no requirement for downstream processes--concentration and freeze-drying --as with the SmF process.

Software Simulation

Enzyme production process using the traditional SmF method and SSC method discussed in the previous section is simulated in *SuperPro Designer 5.5* software (Intelligen, Inc, 2004). The software simulation inputs include operation mode specification, material registration, procedural operations specification, etc. The main window for this software is shown in Figure 6. For detailed software simulation information, see Zhuang's thesis (2004).

Figure 5. The SSC method for producing enzymes –Plant specification

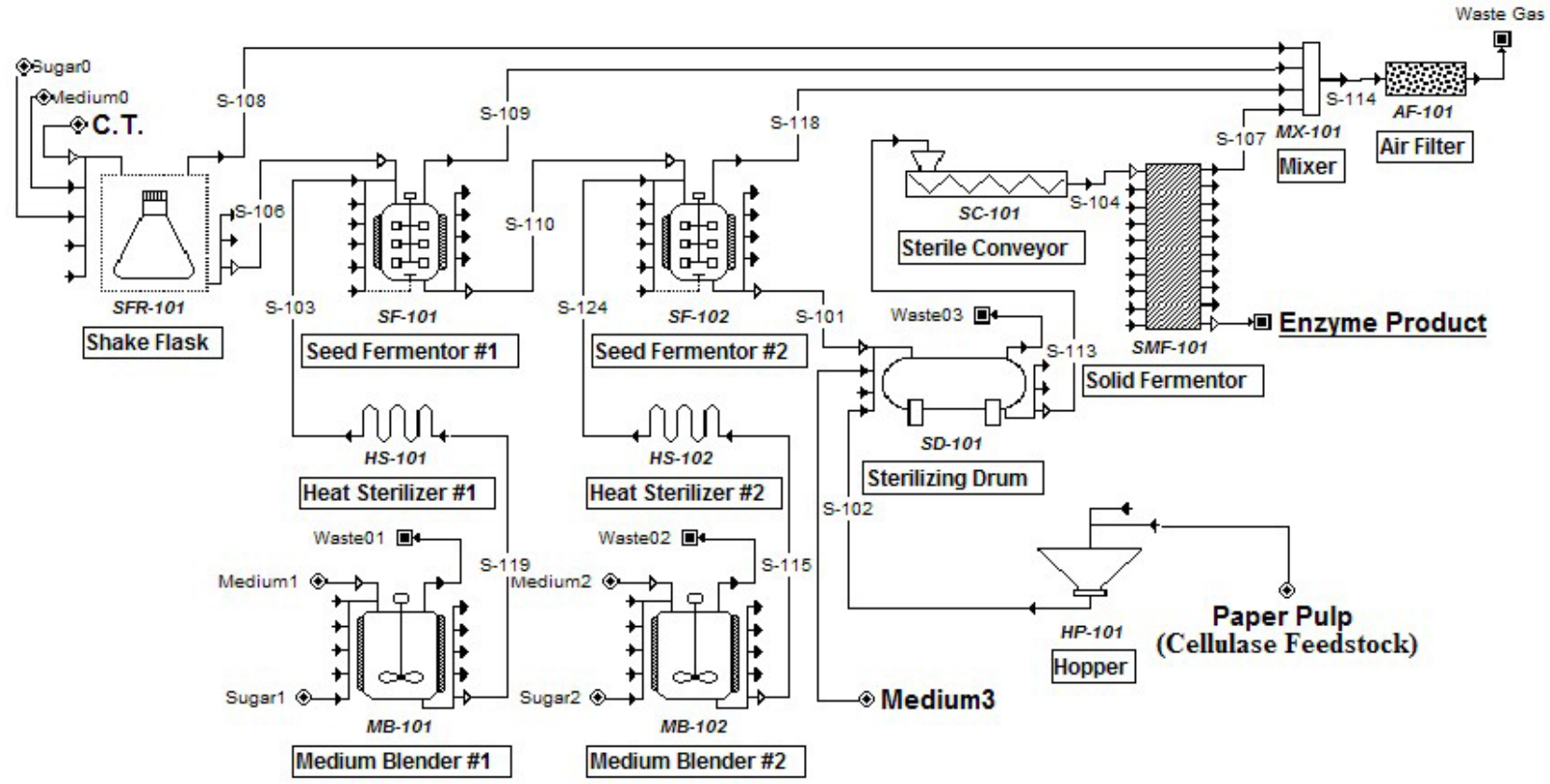
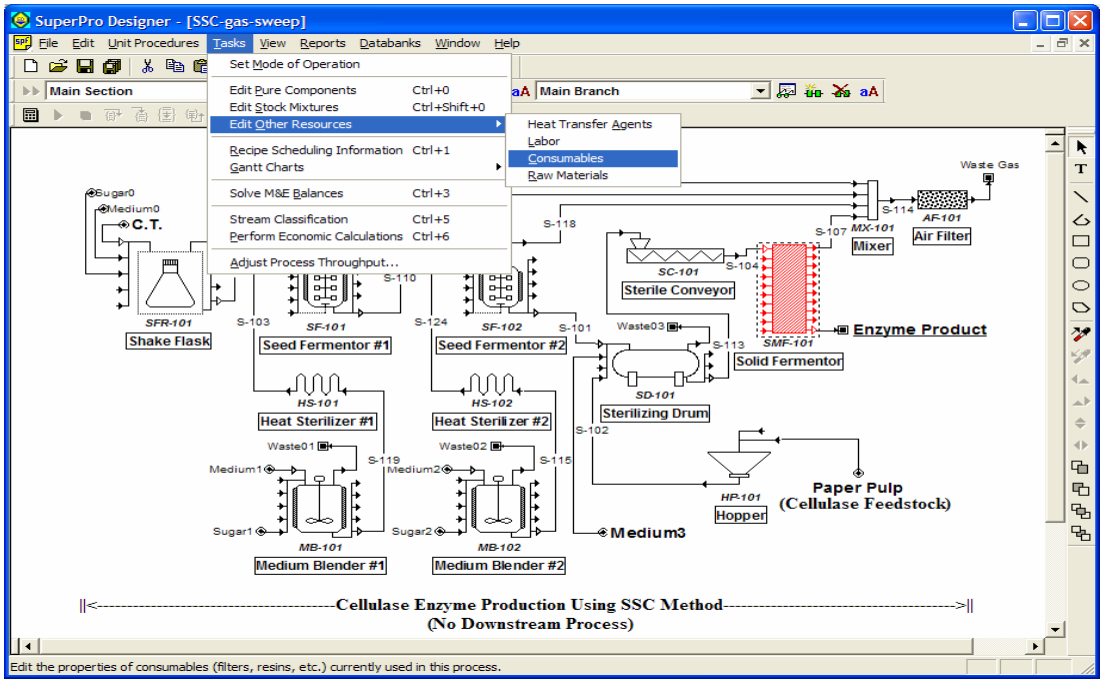


Figure 6. Main window for the *SuperPro Designer 5.5* software



From an economic viewpoint, the input for the fermentation or bacteria growth is the feedstock cellulose. The environment for the fermentation is the medium. And the output for the fermentation is new bacteria, enzymes, and other fermentation end products (FEP). Thus, for economic analysis purposes, a simple mass-balance equation instead of complex equations is used to describe the enzyme production process (Raimbault, 1998), specified below.

As a starting point, the cellulase enzyme production scale from the main fermentor is assumed to be 10,000 kilograms (kg) per batch. Zhang and Lynd (2003) reported that the cellulase enzyme represented 20% of the *C. thermocellum* bacteria mass, which implies 50,000 kg of by-product bacteria ($50{,}000 = 10{,}000 \div 20\%$) will be produced. Based on information obtained from microbiologist Dr. Herbert Strobel (2004), the cellulose-bacteria mass transfer coefficient is assumed to be 10:1, which implies in

order to get 50,000 kg of bacteria, 500,000 kg of cellulose must be consumed. Thus, for every 500,000 kg of cellulose consumed, the final product will be 50,000 kg of new *C. thermocellum* bacteria, 10,000 kg of cellulase enzymes and 440,000 kg of fermentation end products (FEP). Equation 1 represents this simplified fermentation process and provides a basis for economic analysis in this research.

(1) Cellulose → New Bacteria + Cellulase Enzyme + FEP
(500,000 kg) (50,000 kg) (10,000 kg) (440,000 kg)

This research assumes the reaction efficiency is 100%. In order to obtain 10,000 kg of cellulase enzyme, 500,000 kg of cellulose must be provided. In order to obtain 500,000 kg of cellulose, 500,000 kg of cellulose powder (assuming 100% purity at this time) or 914,622 kg of paper pulp ($914{,}622 \approx 50{,}000 \div 0.5456$, considering the mass composition of cellulose in paper pulp is 0.5456) are required as a feedstock for the solid fermentor.

Based on the information discussed above, medium needed are calculated below for the SmF and SSC processes, respectively. (1) For the SmF process, to match this amount of cellulose (500,000 kg), according to Wooley et al. (1999), the initial cellulose concentration is assumed to be 4%. So the medium required for the SmF process is calculated and equals 12,500,000 kg ($12{,}500{,}000 = 500{,}000 \div 4\%$). (2) For the SSC process, to match this amount of paper pulp (914,622 kg), according to Chinn's

dissertation (2003), the moisture content is assumed to be 70%. So the medium required for the SSC process is calculated and equals 2,134,118 kg

($2{,}134{,}118 \approx 914{,}622 \times 70\% \div (1 - 70\%)$).

Bacteria reproduces quickly. It is assumed that the bacteria multiply 100 fold in a shake flask, seed fermentors and fermentors, for the SmF and SSC processes, respectively. The bacteria produced in the previous vessel is the feed for the next vessel. The data for the cellulose, medium, bacteria and cellulase enzymes discussed above are scaled down from the liquid fermentor to seed fermentor #2, from seed fermentor #2 to seed fermentor #1, and from seed fermentor #1 to shake flask, by a factor 0.01, respectively. The data discussed above regarding the mass balance in the vessels in the SmF and SSC processes are represented in Table 1.

Table 1. Mass balance in the vessels in the SmF and SSC processes (kg)

		Shake Flask	Seed Fermentor #1	Seed Fermentor #2	(SmF) Liquid Fermentor	(SSC) Solid Fermentor
Input	*C.T.*	0.0005	0.05	5	500	500
	Cellulose	0.5000	50.00	5,000.0	500,000*	500,000*
	Paper Pulp	N/A	N/A	N/A	916,422	916,422
	Medium	12.5000	1,250.00	125,000.0	12,500,000	2,134,118
Output	**Cellulase Enzyme**	0.0100	1.00	100.0	**10,000**	**10,000**
	C.T.	0.0500	5.00	500.0	50,000	50,000
	FEP	0.4400	44.00	4,400.0	440,000	440,000

*contained in the paper pulp, not from cellulose powder.

Note: (1) C.T. = *C. thermocellum* bacteria; FEP = fermentation end product

(2) Output of C.T. from previous vessel (e.g., shake flask) is the input of the C.T. for the next vessel (e.g., seed fermentor #1);

(3) All the data are based on a starting-point production rate: 10,000 kg of cellulase enzyme per batch from main fermentor;

(4) Reaction efficiency is assumed to be 100%;

These sections discussed above provide key input data for the software simulation. Other input data are omitted in this paper but available in Zhuang's thesis (2004). Based on all the input information, *SuperPro Designer 5.5* software provides simulation results, a basis for the consequent economic analysis and sensitivity analysis.

ECONOMIC ANALYSIS AND SENSITIVITY ANALYSIS

Enzyme production simulations discussed in the previous section has built a user-friendly adaptable computer model for further analysis. When necessary data are obtained and input into the computer model, simulation output will be provided. Economic analyses and sensitivity analyses are conducted in this section to examine: (1) the unit costs to produce enzymes using the traditional submerged fermentation (SmF) method and the solid state cultivation (SSC) method, as measured by dollars per kilogram ($/kg); and (2) the profitability of the experimental enzyme production plant using the SSC method, as measured by three profitability indicators: payback period, net present value and internal rate of return.

For objective one, unit costs are specified by the software simulation output. This allows unit costs comparison between the two methods without considering the revenues associated with the sales of the final enzyme products. For objective two, three profitability indicators--payback period, net present value and internal rate of return--are calculated, using the data for both enzyme production costs and sale revenues.

Unit Cost Analysis

The unit costs for each method of cellulase enzyme production are calculated as the quotient of the annual operating cost divided by the annual enzyme production rate. The enzyme production rate is the product of the output per batch (*OPB*) and the number of batches per year (*NBPY*), shown in equation 2.

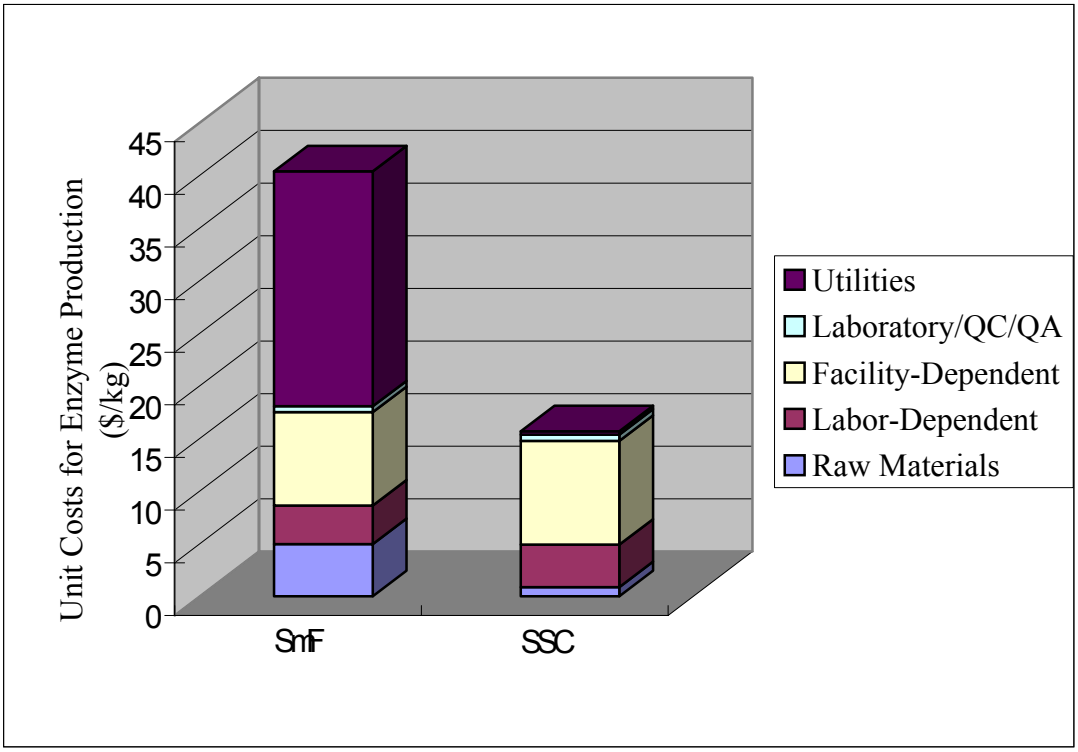
(Equation 2) $$Unit\ \ Cost\ \ (\$/kg) = \frac{Operating\ \ Costs\ \ (\$)}{\Pr oduction\ \ Rate\ \ (kg)} = \frac{Operating\ \ Costs\ \ (\$)}{NBPY \times OPB\ \ (kg)}$$

Using the Equation 2, software calculate the unit costs for enzyme production, which equal 15.67 $/kg for the SSC method and 40.36 $/kg for the SmF method. Unit costs shares are calculated for the SmF and SSC processes, respectively, shown in Table 2 and Figure 7.

Table 2. Itemized unit costs for enzyme production

	SmF		SSC	
Cost Item	**%**	$	**%**	$
Raw Materials	**12.27**	4.95	**5.57**	0.87
Labor-Dependent	**9.07**	3.66	**25.71**	4.03
Facility-Dependent	**22.00**	8.88	**62.87**	9.85
Laboratory/QC/QA	**1.36**	0.55	**3.86**	0.60
Utilities	**55.30**	22.32	**1.99**	0.31
Miscellaneous	**0.00**	0.00	**0.00**	0.00
TOTAL	**100%**	**$40.36**	**100%**	**$15.67**

Figure 7. Unit costs share for enzyme production



Source: Table 2.

Table 2 and Figure 7 indicate that (1) the SSC method is more economical than the SmF method with lower unit costs for enzyme production; (2) the items of input costs for laboratory/QC/QA, facility-dependent, and labor-dependent components of the SSC method are either nearly the same or slightly greater than the SmF method; and (3) utilities and raw materials costs used by the SSC method are much lower than the SmF method, which is the reason why the SSC method is economical compared to the SmF method.

Profitability Analysis

Reduced unit costs information from the SmF to the SSC method discussed in the previous section is valuable for economists, engineers and microbiologists because they are concerned with the long-run industry sustainability. However, potential investors for the experimental enzyme production plants may be more concerned with the profitability of their investment, considering the enzyme final product is sold at the market price. Profitability is typically measured by some indicators such as payback period, net present value (*NPV*) and internal rate of return (*IRR*), calculated using the Equations 3-5 below, where T= the project life; NCF_t = the net cash flow for the year t (t=1…T.); d=the discount rate.

(Equation 3) $$Payback\ \ Period = \frac{Total\ \ Capital\ \ Investment}{Net\ \ \Pr ofit}$$

(Equation 4) $$NPV = \sum_{t=0}^{T} \frac{NCF_t}{(1+d)^t} = NCF_0 + \frac{NCF_1}{(1+d)^1} + \frac{NCF_2}{(1+d)^2} + ... + \frac{NCF_T}{(1+d)^T}$$

(Equation 5)

$$NPV = 0 = \sum_{t=0}^{T} \frac{NCF_t}{(1+IRR)^t} = NCF_0 + \frac{NCF_1}{(1+IRR)^1} + \frac{NCF_2}{(1+IRR)^2} + ... + \frac{NCF_T}{(1+IRR)^T}$$

The payback period, net present value and internal rate of return are calculated and equal 2.75 years, $30,387,000, 35.55%, respectively, for the enzyme production using the SSC method. These indicator values can be compared with corresponding indicator values of alternative projects facing the potential investors. Generally these numbers indicate the SSC method is economical.

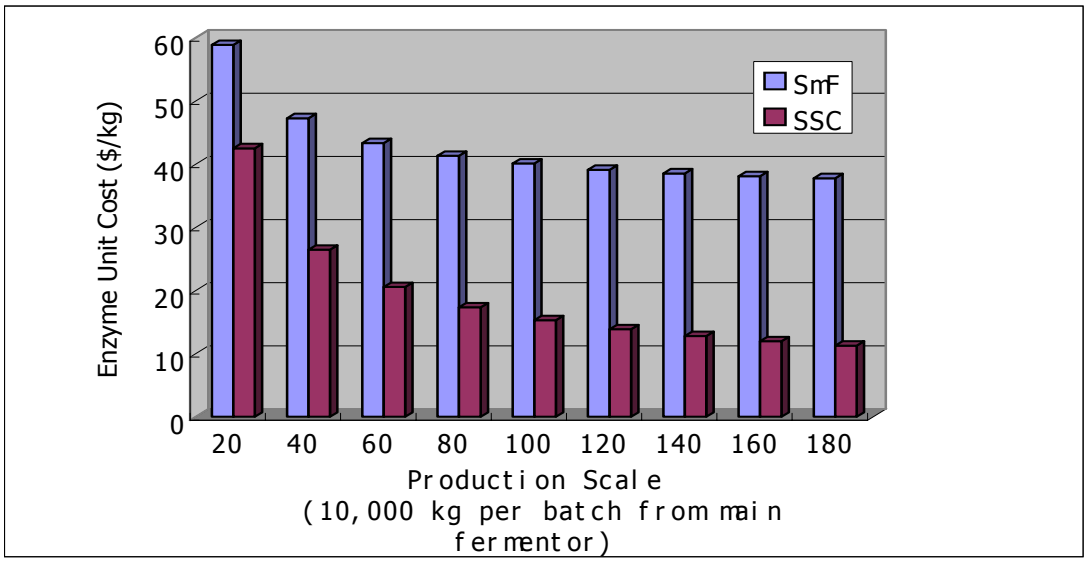
Sensitivity Analysis

In the baseline economic analysis conducted in the previous section, the enzyme production scale is set at 10,000 kilograms of cellulase enzyme per batch from the main fermentors. This number is a starting point and may vary. This section assesses the influence of ±80% change of this initial production scale (-80%, -60%, -40%, -20%, +20%, +40%, +60% and +80%) on the unit costs to produce enzymes (for the SmF and SSC methods) and on the profitability indicators for the simulated enzyme production plants (for the SSC method only). Table 3 summarizes these sensitivity analysis results. Figure 8 presents a comparison of the influence of plant scale changes on the unit costs of enzyme production between the SmF and SSC methods.

Table 3. Sensitivity analyses for the influence of production scale on the unit costs and profitability for enzyme production using the SSC method

Sensitivity Variables		SmF Unit Cost ($/kg)	SSC Unit Cost ($/kg)	Profitability Indicator (SSC only)		
				Payback Period (year)	Net present value ($1000)	Internal rate of return
Production scale: (kg/batch from main fermentor)	-80% (2,000)	58.90	42.51	71.19	-14,636,624	N/A
	-60% (4,000)	47.30	26.46	5.58	-1,539	4.92%
	-40% (6,000)	43.35	20.54	3.79	8,736	14.92%
	-20% (8,000)	41.33	17.34	3.06	20,081	21.64%
	Base (10,000)	40.36	15.67	2.75	30,387	25.39%
	+20% (12,000)	39.12	13.86	2.36	43,869	30.70%
	+40% (14,000)	38.54	12.79	2.16	56,023	33.83%
	+60% (16,000)	38.10	11.95	2.00	68,397	36.64%
	+80% (18,000)	37.77	11.27	1.81	80,955	39.14%

Figure 8. Influence of enzyme production scale on unit costs using the SmF and SSC methods (Using the data from Table 3)



As seen from the Table 3 and Figure 8, the production scale has significant impacts on the unit costs for enzyme production using both the SmF and SSC methods. Also, these results indicate that the SSC method is more economical than the SmF method regardless of production scale changes. As to the influence of production scale changes on the profitability for the SSC method, Table 3 indicates that the SSC method is economical except under the condition that the -80% and -60% changes of the production scales. Thus, sensitivity analysis confirms the profitability of the SSC method.

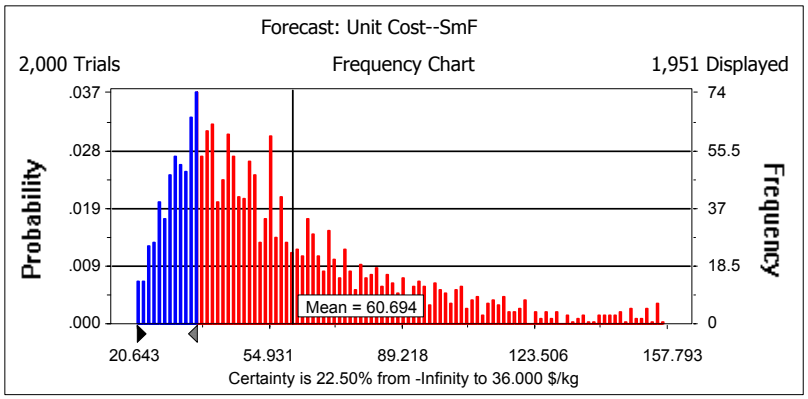
Monte Carlo Analysis

Monte Carlo analysis, a probabilistic method that inputs all variable uncertainties into a model, provides more insight for investors into the unit costs to produce enzymes using the SmF and SSC methods. Figure 9 presents the effects on unit costs for enzyme production using SmF methods, representing all the possible outcomes from random

sampling. Shown in Figure 9 (a), when compared with the enzyme market price ($36.00/kg), Monte Carlo analysis results show that the SmF method is profitable with 22.50% certainty, which implies the probability to achieve a profit (greater than or equal to the market price, $36.00/kg) is 22.50%. The mean unit cost for enzyme production using the SmF method is $60.69/kg, which is 69% higher than the market price ($36.00/kg).

Figure 9. Monte Carlo analysis results: effect on unit costs for enzyme production using the SmF method

(a) the frequency chart



(b) the sensitivity chart

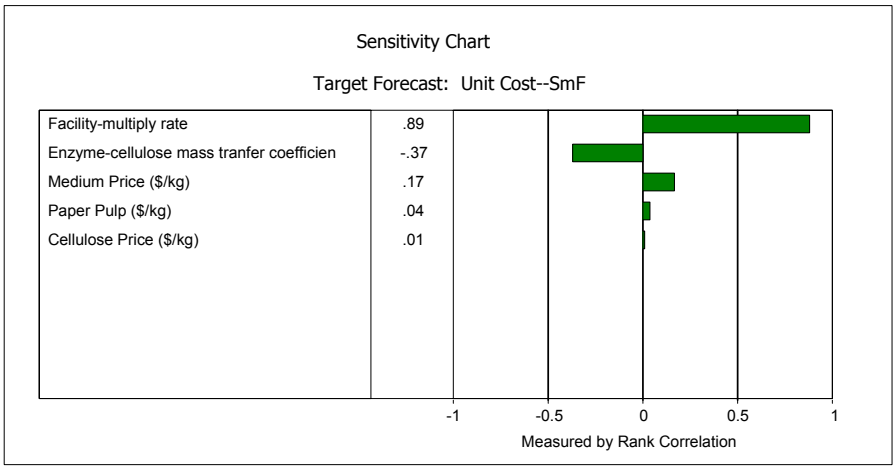
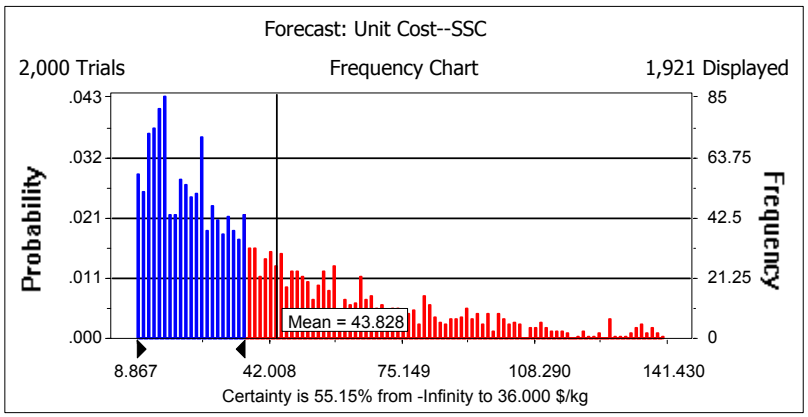
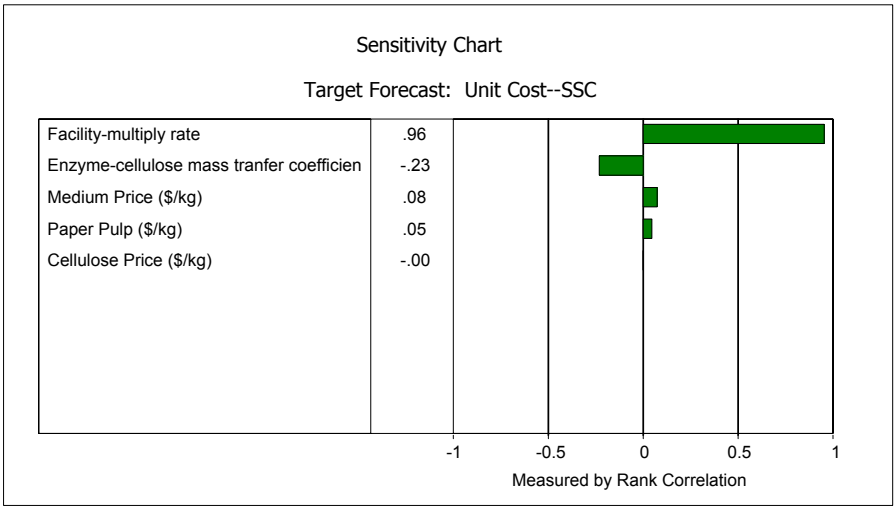


Figure 10. Monte Carlo analysis results: effect on unit costs for enzyme production using the SSC method

(a) the frequency chart



(b) the sensitivity chart



As to the sensitivity of variables, the sensitivity chart (Figure 9 (b)) indicates that **the first and second most influential variables are the facility costs (positive influence) and the enzyme-cellulose mass transfer coefficients (negative influence), respectively.** This implies that a small increase in the facility costs will most increase the unit costs, relatively, while a small increase in the enzyme-cellulose mass transfer

coefficients will most decrease the unit cost, relatively. If researchers can find ways to decrease facility costs (new materials) or increase the enzyme-cellulose mass transfer coefficients (new bacteria), the enzyme production costs may decrease significantly.

By contrast, Figure 10 presents the effect on unit costs for enzyme production using the SSC method, representing all the possible outcomes from random sampling. Shown in Figure 10 (a), when compared with the enzyme market price ($36.00/kg, Monte Carlo analysis results show that the SmF method is profitable with 55.15% certainty, which implies that the probability to achieve a profit (greater than or equal to the market price, $36.00/kg) is 55.15%. The mean unit cost for enzyme production using the SSC method is $43.83/kg, which is 22% higher than the market price ($36.00/kg). Compared with the mean unit cost for SmF method ($60.69/kg), the Monte Carlo analysis confirms that the SSC method is more economical than the traditional SmF method. As to the sensitivity of variables, the sensitivity chart (Figure 10 (b)) for the SSC process is similar to the SmF process. Thus the implications are similar.

SUMMARY AND CONCLUSION

In this paper economic analyses of cellulase enzyme production costs using solid state cultivation (SSC) are performed and compared to the traditional submerged fermentation (SmF) method. Results indicate that the unit costs for the cellulase enzyme production are 15.67 dollar per kilogram ($/kg) and 40.36 $/kg, for the SSC and SmF

methods, respectively, while the market price for cellulase enzyme is 36.00 $/kg. Profitability analysis and sensitivity analysis also provide positive results.

Since these results indicate that the SSC method is economical, ethanol production costs may be reduced, with the potential to make ethanol a viable supplemental fuel source in light of current political, economic and environmental issues.

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