Preliminary plant design of *Escherichia coli* BPPTCC-EgRK2 cell culture for recombinant cellulase production using Oil Palm Empty Fruit Bunch (OPEFB) as substrate

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Abstract. An economic analysis of recombinant cellulase production from *E. coli* BPPTCC Eg-RK2 was conducted to support the fulfilling of Indonesia's energy roadmap for ethanol production. The plant use oil palm empty fruit bunch (OPEFB) as primary substrate in cellulase production, with the expected lifetime of 12 years. The plant is assumed to be built in Indonesia and will fulfill 1% of total market demand. The effect of different pretreatment process (alkaline, steam explosion, and sequential acid-alkaline) on the economic value was also studied. A simulation using SuperPro Designer was used to calculate the mass and energy balance based on the kinetic parameter of *E. coli* BPPTCC-EgRK2. Technology evaluation show that alkaline pretreatment gave the highest yield with no known inhibitors formed. The steam explosion show the lowest lignin and hemicellulose removal and known to form known fermentation inhibitors. The net present value of alkaline, steam explosion, and sequential acid-alkaline pretreatment were USD 7,118,000; - USD 73,411,000 and USD -114,013,000 respectively, which mean alkaline pretreatment is the only economically feasible pretreatment method for recombinant cellulase production.

1. Introduction

Cellulase is enzyme that hydrolyzed cellulose, the most abundant compound in plant cell walls, and widely use in different industry [1]. It is used in ethanol production, denim processing, animal feed additives, and detergents [2, 3]. However, according to Juniarto, as quoted by BPPT in 2013, 99% of the enzymes used for industries in Indonesia are imported from other countries. The import value of enzyme is estimated to reach 121.85 billion rupiah and will continue to increase with a rate of increase of 6.67% per annum. This is unfortunate, as there is enormous potential in Indonesia's natural resources that should be able to meet those needs.

One example of Indonesia's natural resource potential is oil palm [4]. The processing of palm leads to the formation of several by-products and residues that have economic potential [5, 6]. Empty fruit bunches (EFBs) are solid residue and they have a high cellulose content, at 45.80% of EFB dry weight. In this case, the high cellulose content of EFB is used as a substrate for bacteria cultivation to produce cellulase. By using EFB as an alternative substrate, the production cost of cellulase on an industrial scale can be suppressed.

Currently, most cellulase enzymes are produced from saprophytic microorganisms [3], with fungi from *T. reseei* being one of the most used in industrial applications [7]. However, industrial applications often require a cost-effective production of enzymes on a large scale, which is a common bottleneck. An overexpression of individual cellulose enzymes using recombinant DNA is one of the common solutions [3]. *Escherichia coli* and *Bacillus* are two most common microbes used to express recombinant proteins [3, 7]. The feasibility of recombinant cellulase production from *E. coli* will be evaluated in this study using the SuperPro Designer Simulator.

In prior to simulate a cellulase plant design, kinetic studies of bacteria used in cultivation is needed to create an accurate simulation. These studies was done in prior research and used as primary data in this research.

2. Materials and Methods

2.1. Process description

The production of recombinant endo-β-1,4-glucanase is based on kinetic study from this and previous study [8] and the process scheme was shown in Figure 1. Oil palm empty fruit bunch (OPEFB) was used as raw materials for cellulase enzyme production in this study. OPEFB was collected from local palm oil mill and its characteristics were shown in Table 1. The OPEFB was pretreated with three different procedure, alkaline [9], steam explosion [10], and sequential acid-alkaline [11]. The pretreated OPEFB was fermented using *Escherichia coli* BPPTCC-EgRK2. The microbes was obtained from Indonesia's Agency for the Assesment and Application of Technology.

The fermentation was conducted in two seed fermenter and one production fermenter operating in stagered mode. All of the fermenter was regarded as a completely mixed reactor. The seeding fermenter to fermenter ratio was 1:40, with fermentation time of 4-7 h. The optimum production conditions were obtained from previous study at pH 7, agitation at 150 rpm and temperature of 37°C and known to produce 3.5 IU/mL cellulase [8,12]. The known cellulase concentration was used to estimate the scale up enzyme production yield. The fermentation reaction was:

$$C_6H_{12}O_6 + 0.69\ C_{10}H_{14}N_2O_5 + 1.56\ NH_3 + 0.085\ O_2 \rightarrow 12.25\ CH_{1.77}O_{0.49}N_{0.24} + 2.33\ H_2O + 0.64\ CO_2$$

The following assumption is used for the mass balance: 1) nitrogen sources come from fish powder which represent as thymidine, 2) cellulase was intracellular product represent by *E. coli,* 3) glucose, cellulase and cell concentration was calculated based on experimental data.

The resulting cell was separated from its medium using centrifugation to concentrate the cell before disrupted. Cell was disrupted to release its cellulase using high pressure homogenizer at 800 bar with 3 passes. Cellulase was then separated from cell debris using centrifugation. The crude cellulase was then stabilized using maltodextrin [13] and spray dried to produce crude enzyme powder. The spray dried product was expected to have activity loss up to 37%.

2.2. Economic analysis

The mass and energy balance was calculated from SuperPro Designer 9.0 based on the described process. The same simulation was also used to estimate various economic parameters using data such as equipment purchase cost, direct cost and indirect cost. There were two costs used to evaluate the economic parameters of the plant, total capital investment (TCI) and annual operating cost. TCI was calculated based on main equipment purchase cost. The equipment cost was estimated using equation [14] and graph [15]. The additional direct/indirect costs was estimated from the equipment purchase cost [15]. Annual operating cost was estimated from raw materials cost, labor dependent cost, facility dependent cost, utilities and depreciation cost.

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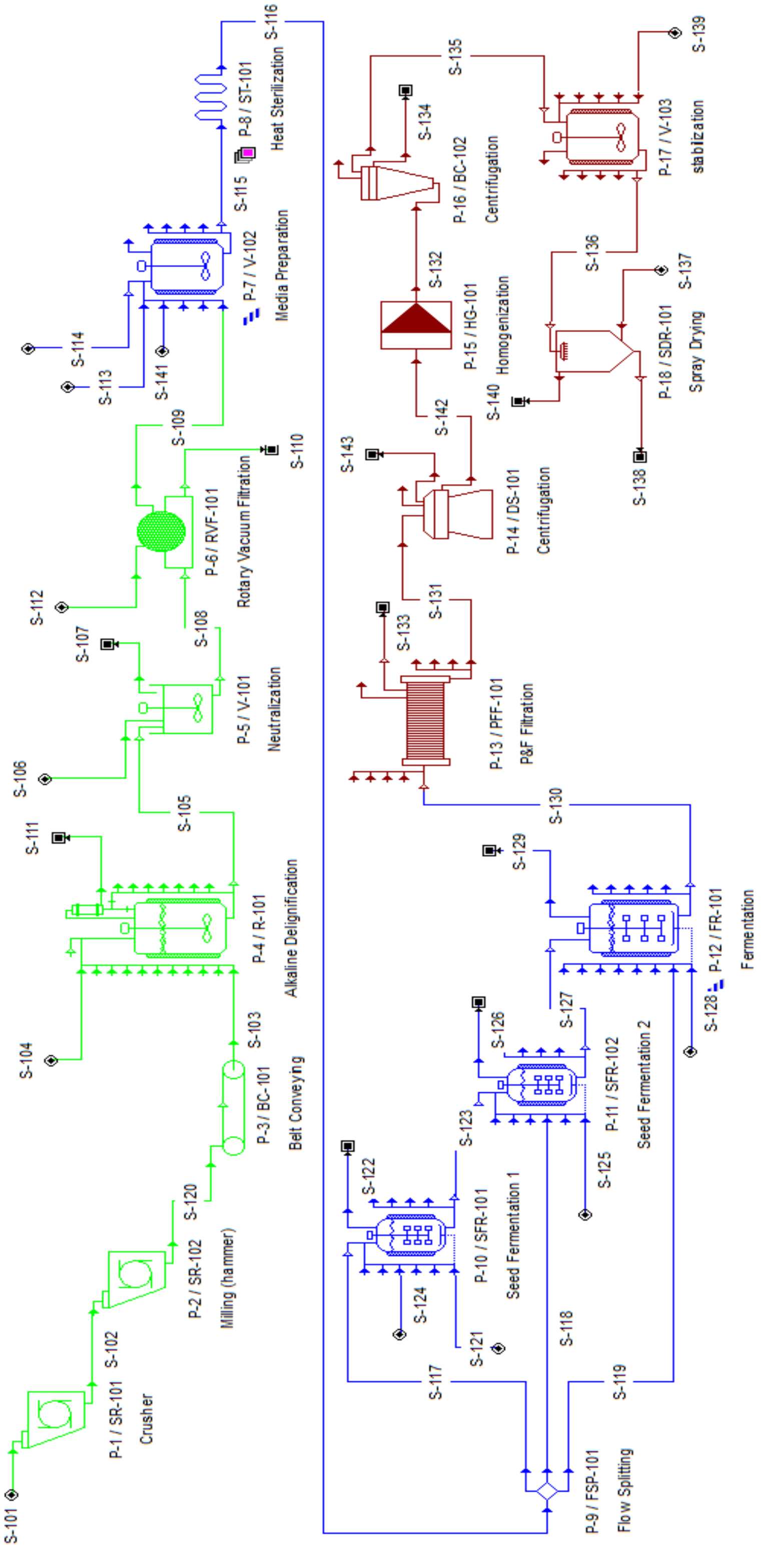

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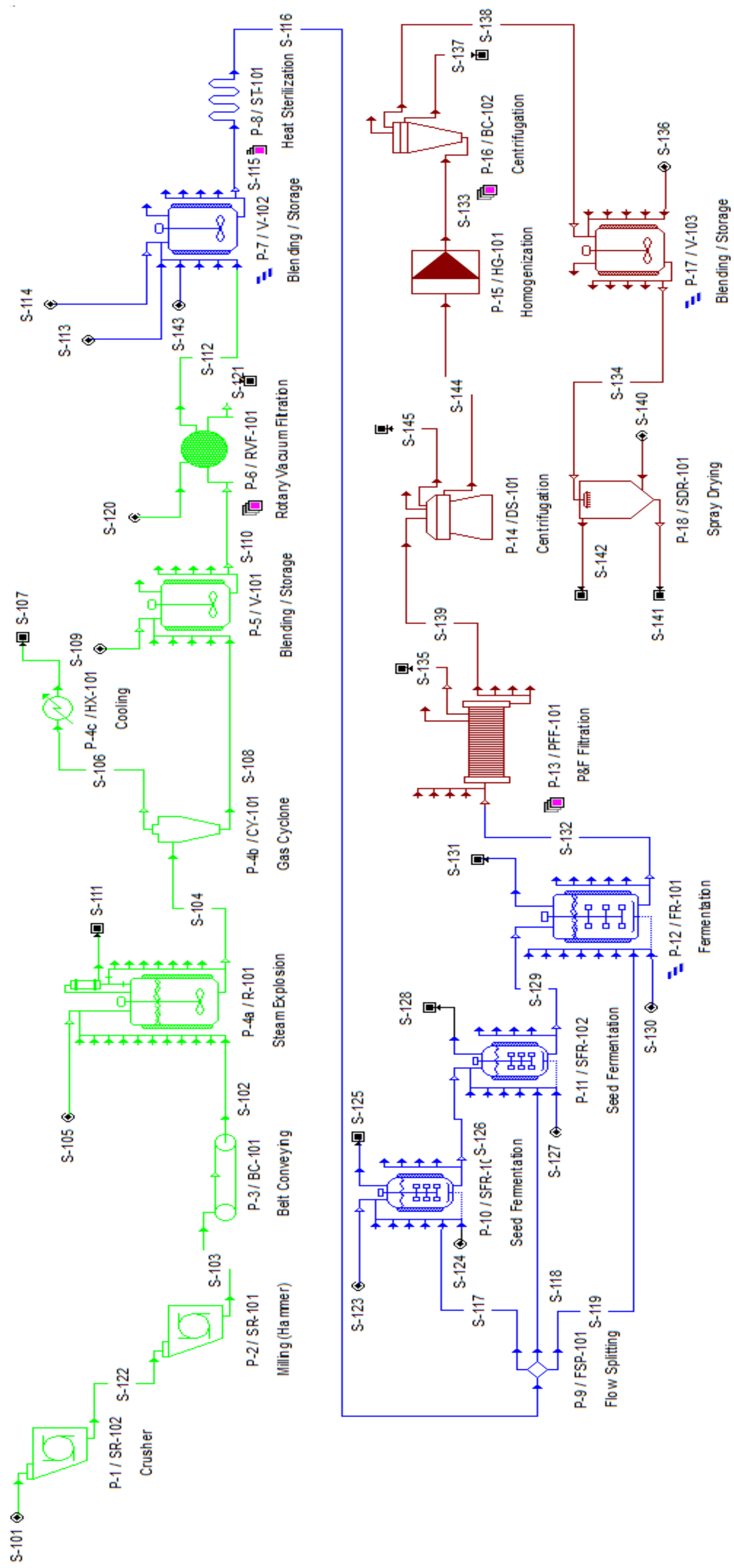

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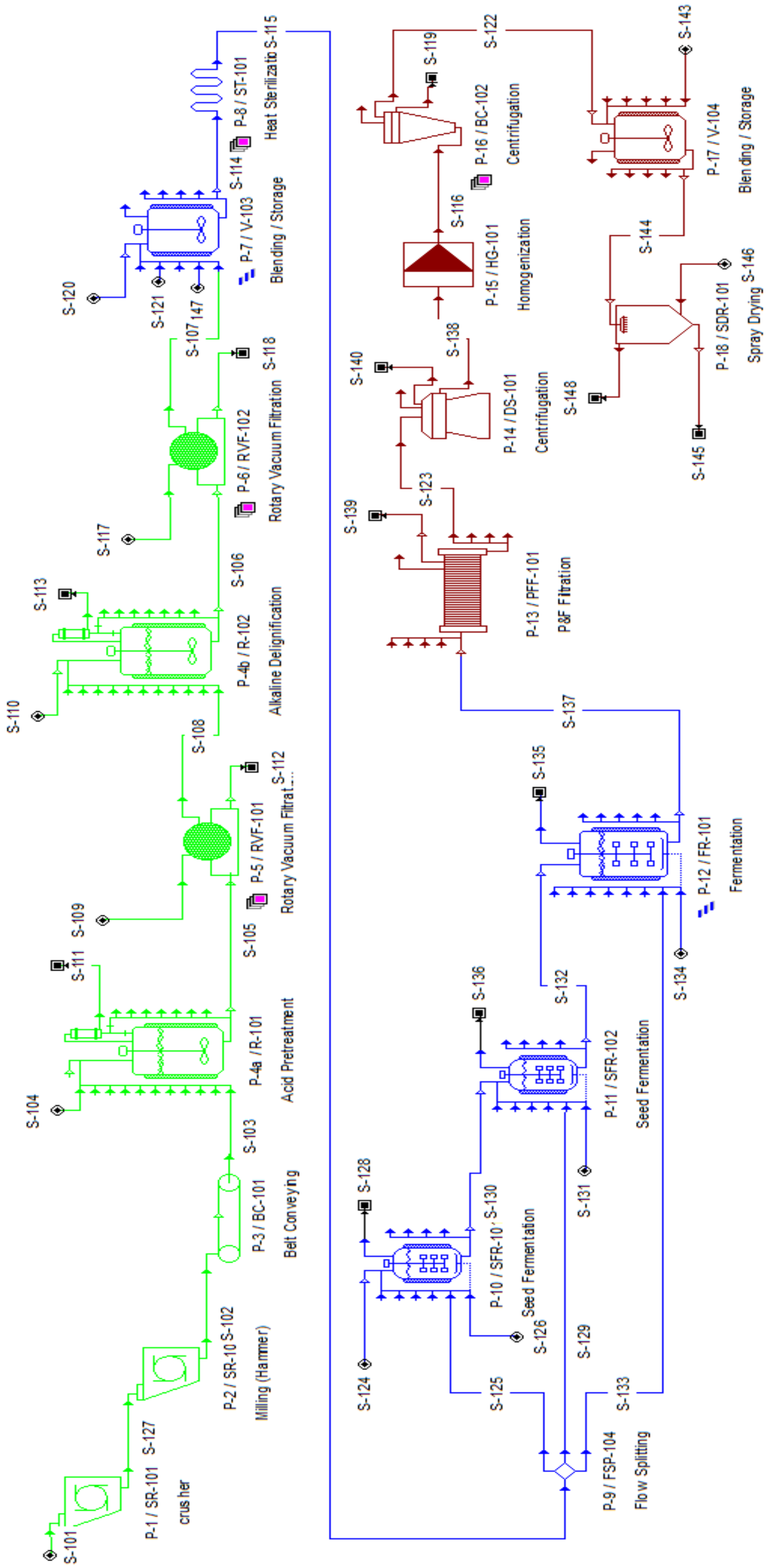

(C)

Figure 1. Cellulase Production Process scheme with A) Alkaline pretreatment, B) steam explosion pretreatment, and C) sequential acid-alkaline pretreatment.

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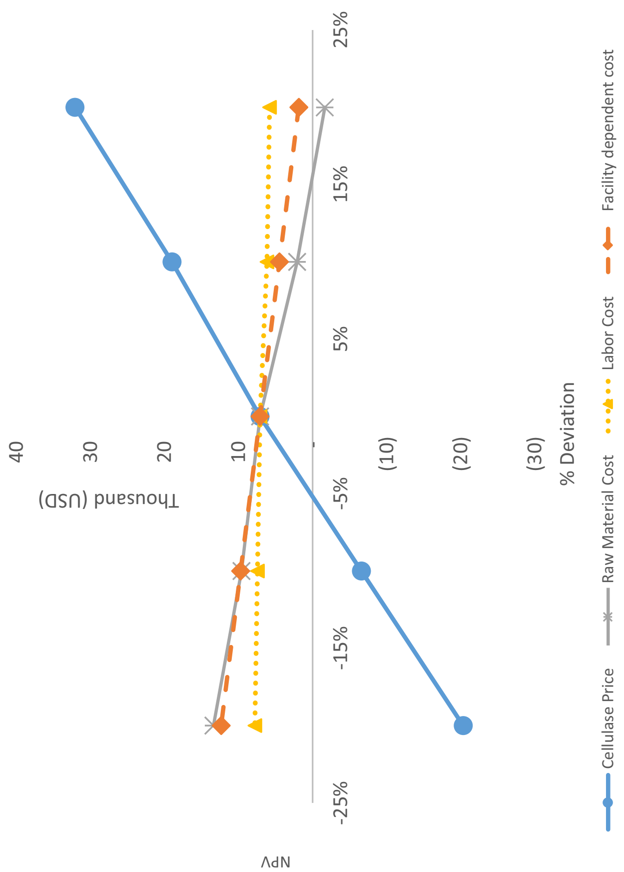

Figure 2. Sensitivity analysis of recombinant cellulase production from OPEFB

3. Results and Discussions

3.1. Techno-Economy analysis of cellulase production

In order to evaluate the most suitable cellulase production process, the yield, batch duration, number of unit, pretreatment performance and economic parameter were compared. The alkaline pretreatment process was able to fulfill 1% of Indonesia's market share using 8.5 ton of OPEFB as substrate, smaller then the acid-alkaline and steam explosion pretreatment which required 19 ton and 29 ton OPEFB, respectively. The alkaline pretreatment also give the highest product yield, 0.03, compared with acid-alkaline and steam explosion, which gives yield of 0.021 and 0.008.

As for delignification efficiency, the sequential acid-alkaline gives the highes lignin removal, by removing 90% lignin from OPEFB [11]. Steam explosion did not remove any lignin from OPEFB but gives high hemicellulose removal, which increase its permeability and increase enzimatic digestibility [10]. However, this process also produce fermentation inhibitors such as levulinic acid (0.11 g/L), formic acid (0.21 g/L), hidroxymethyl furfural (0.71 g/L) and furfural (0.23 g/L) [10]. Similar result also achieved by another study [16]. The performance of each simulation is shown in table 1.

Table 1. Simulation summary of different pretreatment method effect on project profitablility

Parameter	Pretreatment Method		
	Alkaline (CHEMEX) [1]	***Steam Explosion* [2]**	**Acid-Alkaline [3]**
OPEFB Feed/batch (Ton)	8.5	29	19
Yield p/s	0.03	0.008	0.021
Number of units	18	20	21
Fermentation inhibitor	-	Present	-
Removal efficiency			
-Cellulose	19%	30%	41%
-Hemicellulose	67%	82%	86%
-Lignin	80%	0%	90%
Total Capital Investment (USD)	35,195,000	53,227,000	83,383,000
Operating Cost (USD)	23,094,000	34.948,000	41,692,000
Internal Rate of Return	14.61	N/A	N/A
Net Present Value (9.6%) (USD)	7,118,000	-73,411,000	-114,013,000
Pay back period	4,84	N/A	N/A

3.2. Economic analysis

The TCI was calculated based on purchase and instalation cost of each equipment, piping, instrumentation, facility dependent cost, engineering and construction fee. Table 1 show the total capital investment and annual operating cost of each simulation. The TCI was 35,195,000 USD, 53,227,000 USD and 83,383,000 USD for alkaline pretreatment, steam explosion pretreatment and acid-alkaline pretreatment. High capital investment on steam explosion and sequential acid-alkaline pretreatment was due to its number of unit. Steam pretreatment have 20 different unit and sequential acid-alkaline pretreatment used 21 units with multiple rotary vacuum filtration unit, resulting in higher capital investment on equipment and installation cost. The annual operating cost for recombinant cellulase production were highly influence by raw materials cost, labor-dependent cost, and waste treatment cost. Acid-alkaline pretreatment had the highest operating cost at 41,692,000 USD due to energy intensive pretreatment process. Steam explosion also give high operational cost due to low conversion rate, resulting in high raw materials cost and high waste treatment cost.

The plant revenue came solely from cellulase sales. The annual cellulase production was 154 ton (1% market share), with revenue of 30,800,000 USD. NPV was the key to determine the feasibility of a project. Simulation show that alkaline pretreatment gives the highest NPV, 7,118,000 USD. Both steam explosion pretreatment and sequential acid-alkaline pretreatment process gives negative NPV value due to higher operational cost.

3.3. Sensitivity Analysis

Project feasibility is also evaluated from its sensitivity to key variables, such as selling price, raw materials cost and labor cost. A project that highly affected with external factors are considered riskier. Figure 2 show the effect of changes in labor cost, raw materials cost, selling price, and facility dependent cost to NPV value. It was found that cellulase selling price have the highest influence on the NPV. However, as this project was intended to fulfill Indonesia's government renewable energy target, a steady demand is expected in near future. Both labor cost, raw materials cost, and facility dependent cost do not affect the project as much as the selling price, showing less risk on these parameters.

4. Conclusions

In this study, the techno-economic analysis of recombinant cellulase production from OPEFB was investigated. A comparison of three different pretreatment process were evaluate to find the most feasible process. Recombinant cellulase production using alkaline pretreated OPEFB was found to give highest NPV, internal rate of return, and payback period with value of 7,118,000 USD, 14.61% and 4.84 years, respectively. This project also found to be sensitive to cellulase selling price, as it gives highest impact on the net present value.

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