Techno-economic analysis of recombinant Endo-β-1,4-Glucanase production from *Escherichia coli* Eg-RK2 culture using oil palm empty fruit bunch

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**Abstract.** A techno-economic analysis of recombinant cellulase production from *E. coli* Eg-RK2 was conducted to support the fulfilling of Indonesia’s energy roadmap for ethanol production. The plant utilizes OPEFB as a primary substrate in cellulase production, with an expected lifetime of 12 years. The plant is assumed to be built in Indonesia and it will fulfill 1% of the total market demand. The effect of different pretreatment processes (alkaline, steam explosion, and sequential acid-alkaline) on the profitability parameter was also studied. A simulation using SuperPro Designer was used to calculate the mass and energy balance based on the kinetic parameters of *E. coli* EgRK2. A technology evaluation showed that alkaline pretreatment provides the highest yield with no known inhibitors formed. The steam explosion pretreatment offers the lowest rate of lignin and hemicellulose removal, and it is understood to form known fermentation inhibitors. The NPVs of the alkaline, steam explosion and sequential acid-alkaline pretreatments are USD 32,121,000, USD 36,841,000, and USD 384,000, respectively, which means the alkaline pretreatment is economically very feasible for the production of cellulase.

1. Introduction

Cellulases are currently the third global largest industrial enzyme can be used in ethanol production, denim processing, animal feed additives, and detergents [1-3]. Oil palm empty fruit bunch (OPEFB) can have up to 46.77% cellulose content [4]. The high cellulose content of OPEFB can be used as a low-cost substrate for bacteria cultivation to produce cellulase [5]. Studies have been carried to improve the utilization of cellulase from OPEFB or other natural materials including process optimization and immobilization of this enzyme [6-8].

Mostly cellulase are produced from saprophytic microorganisms [2], with fungi from *T. reseei* as the most used in industrial applications [9]. However, industrial applications often require a cost-effective production of enzymes on a large scale, which is a common bottleneck. An overexpression of individual cellulase enzymes using recombinant DNA is one of the common solutions [2]. *Escherichia coli* and *Bacillus* are common microbes to express recombinant proteins [2, 9-11]. The feasibility of recombinant cellulase production from *E. coli* will be evaluated in this study using the SuperPro Designer Simulator.
Prior to simulating the cellulase plant design, kinetic studies of bacteria used in cultivation are needed to create an accurate simulation. In this research, kinetic studies were examined with the Monod approach to get the Monod constant (Ks) and maximum specific growth rate (\( \mu_m \)).

2. Materials and methods

2.1. Process description

The production of recombinant endo-\( \beta \)-1,4-glucanase is based on a kinetic study from this and a previous study [12]. The OPEFB pretreatment scenarios are alkaline [13], steam explosion [14], and sequential acid-alkaline [15]. The pretreated OPEFB was fermented using E. coli EgRK2. All of the fermenters were regarded as completely mixed reactors. The seeding fermenter-to-fermenter ratio was 1:40, with fermentation times of 3 h, 4 h, and 10 h, respectively. The optimum production conditions were obtained from a previous study at pH 7, agitation at 150 rpm, and a temperature of 37°C, and this condition is known to produce 3.5 IU/mL cellulase [11, 12]. The fermentation reaction is following:

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 0.69\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_5 + 1.56\text{NH}_3 + 0.085 \text{O}_2 \rightarrow 12.25\text{CH}_1.77\text{O}_{0.49}\text{N}_{0.24} + 2.33\text{H}_2\text{O} + 0.64\text{CO}_2.
\]

The following assumption is used for the mass balance: 1) nitrogen sources come from fish powder, represented as thymidine; 2) cellulase is an intracellular product and is represented as E. coli; 3) glucose, cellulase, and cell concentration were calculated based on the experimental data. The resulting cell was separated from its medium using centrifugation to concentrate the cell before it is disrupted using a high-pressure homogenizer at 800 bar with three passes. Cellulase was then separated from cell debris using centrifugation. Crude cellulase was then stabilized using maltodextrin [16] and spray dried (activity loss of up to 37%) to produce a crude enzyme powder.

2.2. Economic analysis

The mass and energy balance were calculated using SuperPro Designer 9.0 based on Figure 1. The same simulation was also used to estimate various economic parameters using data, such as equipment purchase cost, direct cost, and indirect cost. The resulting data would be used to assess the feasibility of recombinant cellulase production from OPEFB using E. coli EgRK2.

![Figure 1. Process flow diagram of recombinant endo-\( \beta \)-1,4-glucanase production](image)

2.2.1. TCI

Two costs were used to evaluate the economic parameters of the plant, total capital investment (TCI), and annual operating cost. TCI was calculated based on the main equipment purchase cost, and the equipment cost was estimated using an equation from Seider et al. [17] and a graph from Petrides including the additional direct/indirect costs estimation from the equipment purchase [18].
2.2.2. Operating Cost.
The annual operating cost was estimated from the raw materials cost, labor-dependent cost, facility-dependent cost, utilities, and depreciation cost. Depreciation was calculated using a declining balance method for 12 years. The revenue used for the economic evaluation comes from cellulase sales, where the selling price for cellulase was USD 250/kg or \(1.8 \times 10^{-5}\) USD/IU.

3. Results and Discussion

3.1. Technology comparison of cellulase production

To evaluate the most suitable cellulase production process, the yield, batch duration, number of units, pretreatment performance, and economic parameters were compared. As quoted from Indonesia’s Ministry of Energy and Mineral Resources, the alkaline pretreatment process was able to fulfill 1% of Indonesia’s market share using 8.5 tons of OPEFB as a substrate, smaller than the acid-alkaline and steam explosion pretreatments, which required 19 tons and 29 tons of OPEFB, respectively. The alkaline pretreatment also gives the highest product yield, 0.03, compared with acid-alkaline and steam explosion, which give yields of 0.021 and 0.008, respectively. As for delignification efficiency, the sequential acid-alkaline pretreatment gives the highest lignin removal rate, removing 90% of lignin from OPEFB [15]. Steam explosion did not remove any lignin from OPEFB but offers high hemicellulose removal, which increases its permeability and increases enzymatic digestibility [14]. However, this process also produces fermentation inhibitors, such as levulinic acid (0.11 g/L), formic acid (0.21 g/L), hydroxymethyl furfural (0.71 g/L), and furfural (0.23 g/L) [14]. Similar results were also achieved by another study [19]. The performances of each simulation are shown in Table 1.

3.1.1. TCI and operating cost.
The TCI was calculated based on the purchase and installation cost of each piece of equipment, piping, instrumentation, facility-dependent cost, engineering, and construction fee [20]. Table 1 shows the TCI and annual operating cost of each simulation. The main equipment purchasing cost of each piece of equipment was USD 5,213,000, USD 7,948,000, and USD 12,908,000 for the alkaline, steam explosion, and acid-alkaline pretreatments, respectively. The comparison of each equipment cost was shown in Table 2. The annual operating cost of recombinant cellulase production is highly influenced by three different costs: raw materials cost, labor-dependent cost, and facility-dependent cost. These costs were simulated using SuperPro Designer and they are shown in Table 1 and Table 2. The steam explosion pretreatment had the highest operating cost, at USD 36,156,000, with 44.48% spent on labor-dependent costs.

3.1.2. Profitability parameters
The plant’s revenue came solely from cellulase sales. The annual cellulase production was 154 tons (1% market share), with a revenue of USD 37,300,000. The product was sold at USD 250/kg, or \(1.8 \times 10^{-5}\) USD/IU. The price was determined from the prices of other similar products available on the market. Net present value (NPV) was the key to determining the feasibility of a project. Simulations show that the alkaline pretreatment gives the highest NPV, at USD 32,121,000, calculated at a discounted rate of 9.6% and a project lifetime of 15 years. The profitability parameters of other processes can be seen in Table 1.

3.2. Sensitivity Analysis
Project feasibility is also evaluated based on its sensitivity to key variables, such as selling price, raw materials cost, and labor cost. A project that is highly affected by external factors is considered riskier. Figure 1 shows the effect of changes in labor cost, raw materials cost, selling price, and overall operating cost on NPV value.
Table 1. Simulation summary of different pretreatment method effect on project profitability.

| Parameter                        | Alkaline (CHEMEX) [8] | Steam Explosion [10] | Acid-Alkaline [11] |
|----------------------------------|-----------------------|----------------------|--------------------|
| OPEFB feed/batch (Ton)           | 8.5                   | 29                   | 19                 |
| Product/batch (kg)               | 255                   | 231.36               | 394.84             |
| Number of batch/year             | 585                   | 589                  | 428                |
| Yield p/s                        | 0.03                  | 0.008                | 0.021              |
| Number of units                  | 18                    | 20                   | 21                 |
| Fermentation inhibitor           | -                     | Yes                  | -                  |
| Removal efficiency               |                        |                      |                    |
| -Cellulose                       | 19%                   | 30%                  | 41%                |
| -Hemicellulose                   | 67%                   | 82%                  | 86%                |
| -Lignin                          | 80%                   | 0%                   | 90%                |
| Batch duration (h)               | 81.1                  | 174.08               | 85.71              |
| Minimum cycle time (h)           | 13.42                 | 12                   | 18.33              |
| TCI (USD)                        | 35,165,000            | 53,335,000           | 85,493,000         |
| Operating Cost (USD)             | 23,408,000            | 36,156,000           | 34,848,000         |
| IRR                              | 31.64%                | N/A                  | 7.11%              |
| NPV (9.6%) (USD)                 | 32,121,000            | -36,841,000          | 384,000            |
| ROI                              | 37.26                 | 8.02                 | 14.06              |
| Payback period                   | 3.29                  | 12.48                | 7.11               |

Table 2. Summary of estimated equipment cost in 3 scenarios

| Equipment (Unit)                        | Alkaline Pretreatment | Steam Explosion Pretreatment | Acid-Alkaline Pretreatment |
|----------------------------------------|----------------------|------------------------------|---------------------------|
| Crusher (MT/h)                         | Spec. 11.33 F.O.B. 31.0 | Spec. 29 F.O.B. 82.0 | Spec. 19 F.O.B. 49.0 |
| Hammer mill (MT/h)                     | Spec. 8.5 F.O.B. 32.0 | Spec. 29 F.O.B. 71.0 | Spec. 19 F.O.B. 59.0 |
| Conveyor (ft)                          | Spec. 150 F.O.B. 75.0 | Spec. 150 F.O.B. 75.0 | Spec. 150 F.O.B. 75.0 |
| Alkaline delignification (m³)          | Spec. 46 F.O.B. 154.0 | Spec. 181.98 F.O.B. 329.0 |
| Neutralization tank (m³)               | Spec. 46 F.O.B. 68.0 | Spec. 29.63 F.O.B. 235.0 |
| Steam explosion tank (m³)              | Spec. 29.63 F.O.B. 235.0 |
| Gas cyclone (ft³/min)                  | Spec. 15.7 F.O.B. 3.0 |
| Condenser (ft²)                        | Spec. 103.94 F.O.B. 16.0 |
| Washing tank (m³)                      | Spec. 76.68 F.O.B. 68.0 |
| Acid pretreatment (m³)                 | Spec. 214.6 F.O.B. 333.0 |
| Rotary vacuum filter (ft²)             | Spec. 684 F.O.B. 558.0 |
| Media preparation (m³)                 | Spec. 73.18 F.O.B. 107.0 |
| Autoclave (m³)                         | Spec. 73.18 F.O.B. 148.0 |
| Seed fermenter 1 (L)                   | Spec. 42 F.O.B. 2.0 |
| Seed fermenter 2 (m³)                  | Spec. 2.05 F.O.B. 20.0 |
| Production fermenter (m³)              | Spec. 82.27 F.O.B. 179.0 |
| Plate & frame (m³)                     | Spec. 30.32 F.O.B. 153.0 |
| Centrifuge 1 (m³/h)                    | Spec. 15.9 F.O.B. 588.0 |
| High pressure homogenizer (m³/h)       | Spec. 4.9 F.O.B. 71.0 |
| Centrifuge 2 (m³/h)                    | Spec. 13.2 F.O.B. 313.0 |
| Stabilization tank (m³)                | Spec. 7.3 F.O.B. 6.0 |
| Spray dryer (m³)                       | Spec. 4.93 F.O.B. 771.0 |

Spec. = Specification; F.O.B. = free on-board price (in Thousand US dollars).
Figure 1. Sensitivity analysis of recombinant cellulase production from OPEFB

The cellulase selling price has the highest influence on NPV. Both the labor and raw materials costs have similar effects on the project’s NPV. However, the fluctuating costs of these two parameters do not affect the project as much as the selling price, showing less risk related to these parameters.

3.3. Techno-economic analysis

Both steam explosion pretreatment process and sequential acid-alkaline process had higher capital investment then the alkaline process. A high capital investment in steam explosion and sequential acid-alkaline pretreatments was due to its number of units. Steam pretreatment has 20 different units, with nine saccharification tanks, one fermenter, and one stabilization tank standing by in staggered mode. Similarly, sequential acid-alkaline pretreatment uses 21 units with multiple rotary vacuum filtration units, resulting in a higher capital investment in equipment and installation costs. Moreover, both the steam explosion and sequential acid-alkaline pretreatments require more space for their equipments, affecting the land and building costs of the plant.

The steam explosion process gives the highest operating cost. The cost is spent mostly on labor-dependent cost, affected by the high number of units installed and longer process time. The steam explosion has a 5 days saccharification process in 10 different saccharification tanks, resulting in higher number of operators needed. Sequential acid-alkaline process also give higher operating cost then alkaline pretreatment due to higher energy demand for both acid and alkaline processes in longer period of time than the alkaline process.

From the revenue wise, the alkaline pretreatment also give the highest NPV value, showing the investment value in present worth manners. An economically feasible project should give positive NPV value. Sequential acid-alkaline pretreatment gives a positive NPV value but has a payback period more than half of the plant operating time with IRR value smaller than the discounted rate used in NPV calculation. High annual operating cost in steam explosion pretreatment and sequential acid-alkaline pretreatment are reducing the annual profit, resulting in lower rate of return for the investors. A longer project lifetime or lower operating cost are required to make both steam explosion pretreatment and sequential acid-alkaline pretreatment more economically feasible.

4. Conclusion

This study shows that alkaline pretreatment is the most feasible to produce recombinant cellulase from OPEFB. It has the lowest capital investment and annual operating costs, and offer highest investment return, with NPV, IRR, and payback period of 32,121,000 USD, 31.64% and 3.29 years, respectively. These are financially promising figures as it offers higher investment return than Indonesia’s market rate of return. Sensitivity analysis shows that the cellulase selling price has the highest influence on NPV, affecting this value more than other parameters, as it is the only revenue stream at the plant. However, to fulfill the Indonesian government’s renewable energy target, a steady demand is expected in the near future. Further assessment in cellulase purification and stabilization is recommended to find a more cost-effective production method in the future. Financial figures might be different for other regions. Nevertheless, this study offers an advisable background.
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