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# Techno-Economic Evaluation of Hyaluronic Acid Drugs Production Through Repeated Batch Fermentation Method using *Streptococcus zooepidemicus*

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**ABSTRACTS**

Hyaluronic acid $(C_{14}H_{21}NO_{11})n$ is one of the drugs components, a natural polysaccharide compound that makes up connective tissue and is soluble in water. HA can be applied in cosmetic, biomedical, and food industries. The purpose of this study was to determine the feasibility of a hyaluronic acid manufacturing project through repeated batch fermentation using Streptococcus zooepidemicus and then evaluating from an engineering and economic perspective. Engineering analysis was analyzed based on the available tools and raw materials in online web. The economic analysis was done using several economic parameters, such as the length of time required for an investment to return the total initial expenditure (PBP), the condition of a production project in the form of a production function in years (CNPV), project benefits and so on. The results showed that the production of Hyaluronic Acid nanoparticles was very prospective. Technical analysis to produce 86.4 g/liter of HA nanoparticles per day shows the total cost of the equipment purchased to be 7,264.51 USD. PBP analysis shows that investment will be profitable after more than three years. This project can compete with PBP capital market standards because of the short investment returns. To ensure the feasibility of a project, the project is estimated from ideal conditions to the worst case in production, including labor, sales, raw materials, utilities, and external conditions. The analysis concluded that the present project for the production of Hyaluronic acid nanoparticles is prospective in small scale industry and profitable (by positive values in all economic parameters).



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## 1. INTRODUCTION

Hyaluronic acid $(C_{14}H_{21}NO_{11})n$, or known as HA, is a natural polysaccharide compound that makes up connective tissue and is soluble in water. These compounds can be found in living things such as mammals, marine animals, microbes and even in the human body. HA can also be produced from various chemical processes such as isolation, extraction, fermentation, separation and also purification [Maharani et al., 2021].

In the last few years, HA from microbial fermentation has received more attention than extraction from animal sources which can cause cross-species viral infection [Huang *et al.,* 2008]. HA fermentation is mostly carried out by Streptococcus spp., of which HA is a capsular biopolymer spilled onto the medium [Mausolf *et al.,* 1990]. The development of an economical process for mass production is needed to help the competence of the fermentation process [Huang *et al.,* 2008]. HA has significant structural, rheological, physiological, and biological functions. With its characteristic moisture retention and viscoelasticity, coupled with its lack of immunogenicity and toxicity. HA can be applied in cosmetic, biomedical, and food industries [Chong *et al.,* 2005].

Various fermentation modes, such as batch, repeated batch, fed-batch, and continuous culture have been used for HA production [Blank *et al,* 2005; Chen *et al,* 2009; Im JH *et al,* 2009; Liu *et al,* 2008; Vázquez *et al*, 2010; Huang *et al,* 2008; Cooney *et al,* 1999; Don *et al,* 2010]. Most reports on HA fermentation are based on batch culture [Armstrong *et al*, 1997; Huang *et al.*, 2006; John *et al.*, 1994; Kim *et al.*, 1996; Van de Rijn *et al*., 1980]. However, batch culture has a long turnaround time, which greatly reduces the volumetric production rate, and requires high fixed costs per unit product [Huang *et al*., 2008]. Recently, Repeated batch culture has also been used for HA production, and HA productivity has increased significantly [Chen *et al,* 2009; Huang *et al.,* 2008], due to missed completion times and lag phases [Huang *et al,* 2008]. HA has been successfully produced on an industrial scale with Streptococcus sp. as a major producer with lower production costs and less environmental pollution [Chien & Lee, 2007a,b; Fong chong & Nielsen, 2003a,b; Liu *et al,* 2008].

Therefore, the most appropriate method to analyze the economic evaluation is repeated batch fermentation of Streptococcus sp. conducted by Huang et al. The process is shown in **Figure 1.**

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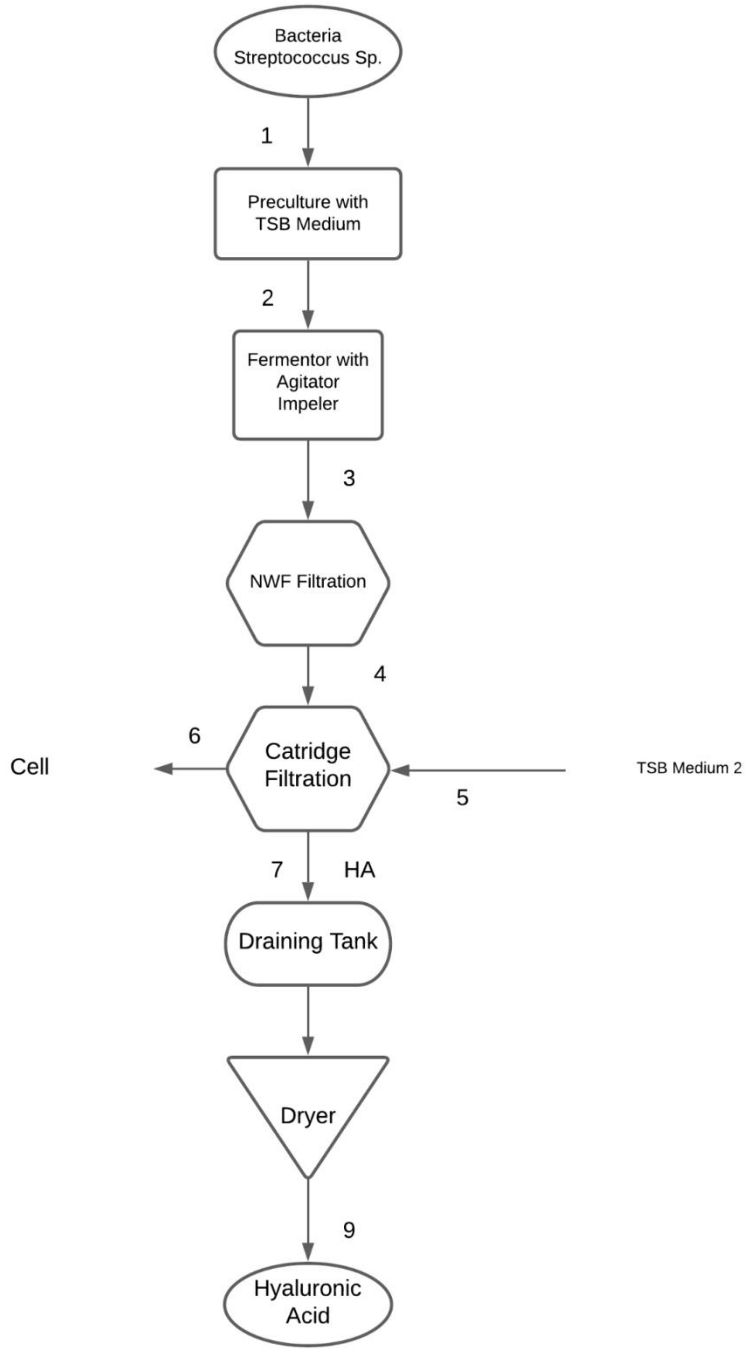


**Figure 1. Synthesis process of HA using fermentation of Streptococcus Sp**

The purpose of this study was to determine the feasibility of a hyaluronic acid manufacturing project through repeated batch fermentation using Streptococcus zooepidemicus and then evaluating from an engineering and economic perspective. The change from lab scale to industrial scale on the quantity of raw materials and equipment was carried out for this research. We perform several economic variations on taxes, sales, raw materials, labor and utilities.

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## 2. MATERIAL AND METHODS

This research uses a method based on the analysis of the price of materials, equipment, and equipment specifications that are commercially available on the online shopping web. All data is calculated based on simple mathematical calculations using Microsoft Excel. The economic evaluation of the project is confirmed by the economic evaluation parameters. There are several economic evaluation parameters used based on literature. [Garret, 2012; Nandiyanto, 2018].

Economic evaluation parameters that are presented in this paper are: (1) Gross profit margin (GPM) is an analysis to determine the level of profitability of a project by reducing the cost of product sales with the cost of raw materials. (2) Payback period (PBP) is a calculation performed to predict the time required for an investment to repay the initial expenditure. PBP is calculated when the CNPV is at zero for the first time. (3) Break even point (BEP) is the minimum amount of product that must be sold at a certain price to cover the production costs used. BEP can be calculated by calculating the value of fixed costs divided (total selling price minus total variable costs). (4) Cumulative net present value (CNPV) is a value that predicts the condition of a production project in the form of a production function in several years. CNPV is obtained by adding the NPV value from the first project to the end of the plant operation. (5) Profitability Index (PI) is an index to identify the relationship between project costs and impacts. PI can be calculated by dividing the CNPV by the total investment cost (TIC). If the PI is more than one, then the project can be classified as a profitable project and if the PI is less than one then the project can be classified as an unprofitable project.

Some assumptions based on the process are found in this paper. This assumption represents a stoichiometric calculation after scaling up the project which yields approximately 86.4 g/L of hyaluronic acid. The assumptions are: (1) All materials used are of high purity. (2) The conversion rate for the hyaluronic acid formation process is 100%.

Several assumptions are used to confirm the economic analysis. These assumptions are used to analyze and predict the possibilities that occur during the project. The assumptions are: (1) All analysis is in USD. 1 USD = 14,460 rupiah [Bank Indonesia, 2021]. (2) Based on the prices of commercially available materials, prices for glucose, K2HPO4, MgSO4 7H2O, agar, soytone, d-glucuronic acid w, glycerol, NaOH 5N, S . zooepidemicus ATCC 39920, and tryptone are 0.73 USD/kg, 0.05 USD/kg, 90.45 USD/kg, 10.50 USD/kg, 80.50 USD/kg, 0.35 USD/kg, 0.88 USD/kg, 48.43 USD/kg, 376 USD/ kg, and 62 USD/kg. The quantity of material is estimated based on stoichiometry. (3) The total investment cost (TIC) is calculated based on the Lang Factor [Garret, 2012]. (4) The TIC is prepared in two steps. The first step is 40% in the first year and the second step is the remainder (during project development). (5) Land purchased. Thus, land costs are added at the beginning of the plant construction year and recovered at the end of the project. (6) Depreciation is estimated using direct calculation [Garret, 2012]. (7) Shipping costs are borne by the buyer. (8) Hyaluronic acid is priced at 4 USD/pack. (9) A one-year project is 240 days (and the rest are days spent cleaning and organizing the process). (10) To simplify utility, utility units can be described and converted as electrical units, such as kWh [Nandiyanto, 2018].Then, the units of electricity are converted into costs. The assumed utility cost is 30.1970 USD/kWh (5 days). (11) Total wages/labor is assumed to be fixed at 120 USD/day for 15 workers. (12) The discount rate is 15% per annum. (13) Income tax is 10% per year. (14) The duration of the project operation is 5 years. Economic evaluation is carried out to test the feasibility of a project. This economic evaluation is done by varying the value of taxes, sales, raw materials, labor and utilities under several conditions. Tax variations are

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carried out at 10, 25, 50, 75 and 100%. Variations in sales, raw materials, labor and utilities were carried out at 80, 90, 100, 110 and 120%.

## 3. RESULTS AND DISCUSSION

### 3.1. Engineering Perspective

**Figure 2** shows the process of making Hyaluronic Acid (HA) using the fermentation broth based on literature. To gain HA, it can be synthesized by isolated the bacteria Streptococcus Sp. using the TSB medium at 37°Cfor 12 hours, then do the fermentation with NWF and cartridge filtration.

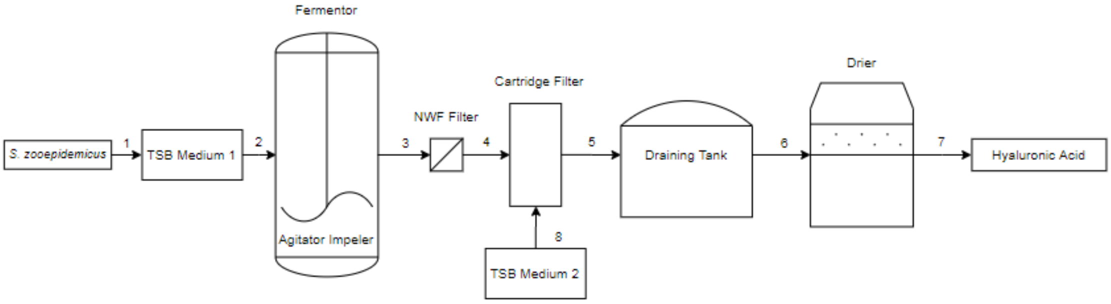


**Figure 2. Process Flow diagram of Hyaluronic Acid**

From an engineering point of view, the total cost for purchasing raw materials for one year is 91,269.92 USD. Sales in one year were 192,000 USD. The profit earned was $ 33,851.02 USD. The price for the analysis of equipment costs is $ 7,264.51 USD. The TIC must be less than $ 7,773.03 USD. This project requires quite a big investment fund and PBP has been reached in the 2nd year.

### 3.2. Economic Evaluation

#### 3.2.1 Ideal Condition

**Figure 3** shows a graph of the relationship between the CNPV / TIC value on the y-axis and the lifetime (year) on the x-axis. n the y-axis which is CNPV / TIC (%) will have a negative value in the first year to the second year. This may be caused by a new company that is developing its business so that it has not yet received a return on investment. The lowest CNPV/TIC value occurred in the second year, which was -0.9746 as shown in **table 1.** But right after the second year to the third year, the company got a return on investment and in the following years there was a very drastic increase. This drastic increase means the company's profits. It can be seen that in the third year the value generated from CNPV/TIV was 15,884 which was very different from the second year and so on until the fifth year, the company continued to earn huge profits until it reached a value of 57,553.

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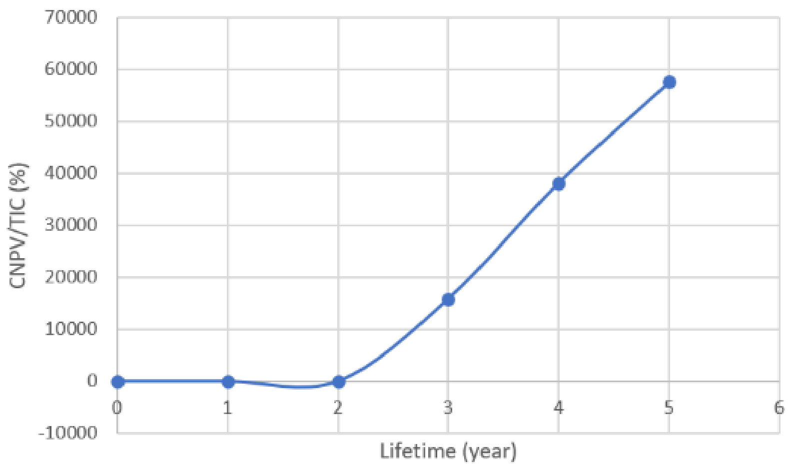


**Figure 3. CNPV/TIC graph for lifetime under ideal conditions.**

**Table 1. Annual CNPV / TIC vallues in ideal conditions.**

| CNPV / TIC | Lifetime (year) |
|---|---|
| 0 | **0** |
| -0.5916 | **1** |
| -0.9746 | **2** |
| 15.884 | **3** |
| 38.172 | **4** |
| 57.553 | **5** |

### 3.2.2 The Effect Of External Conditions

**Figure 4** shows that the company will not benefit at all if it gets a tax of 100%. Meanwhile, if the company gets a tax of 10%, 25% and 75% there will be a return on investment in the second year. Although variable taxes vary, hyaluronic acid production remains quite profitable. Judging from the graph, the most profitable tax value for the company is 10%. Funds that will return when taxes have to be paid according to the 10% PBP analysis will be achieved in the 3rd year and continue to earn big profits in the following year. Therefore, the maximum tax earned for obtaining the BEP or the point at which the project's profit or loss is 75%. If the tax change earned is more than 75%, it can make the project fail.

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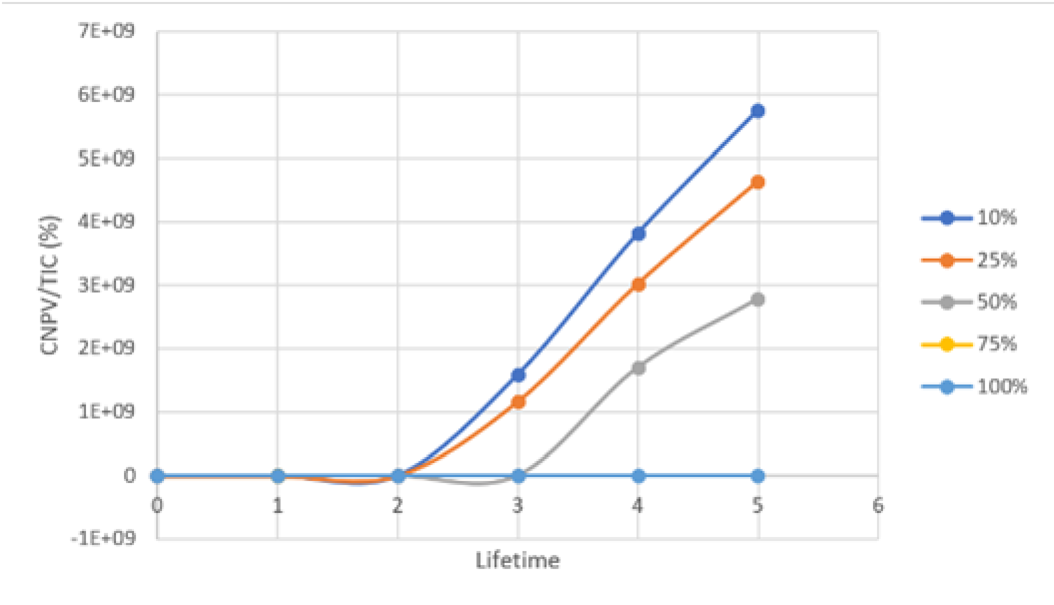


**Figure 4. Graph of CNPV/TIC for tax variations**

### 3.2.3 Change in Sales

**Figure 5** shows a CNPV chart with various sales variations. Sales variations have a very diverse impact on the company. **Figure 5** shows the ideal sales is 100%, when sales are decreased by 20%, the sales 80% of the ideal 100%, respectively and there will be continuous loss until the fifth year. But, If sales are made to 90%, then the company will experience a return on investment after 3.9 years and profits obtained after the fourth year even though the value is very low. When sales are increased by 10% and 20%, the sales will be 110% and 120%. However, if sales are varied to 110%, the company becomes unstable because it fluctuates between the second and fifth years. However, the company will continue to experience profits and return on investment occurs in the second year if the sales variation is 120%.

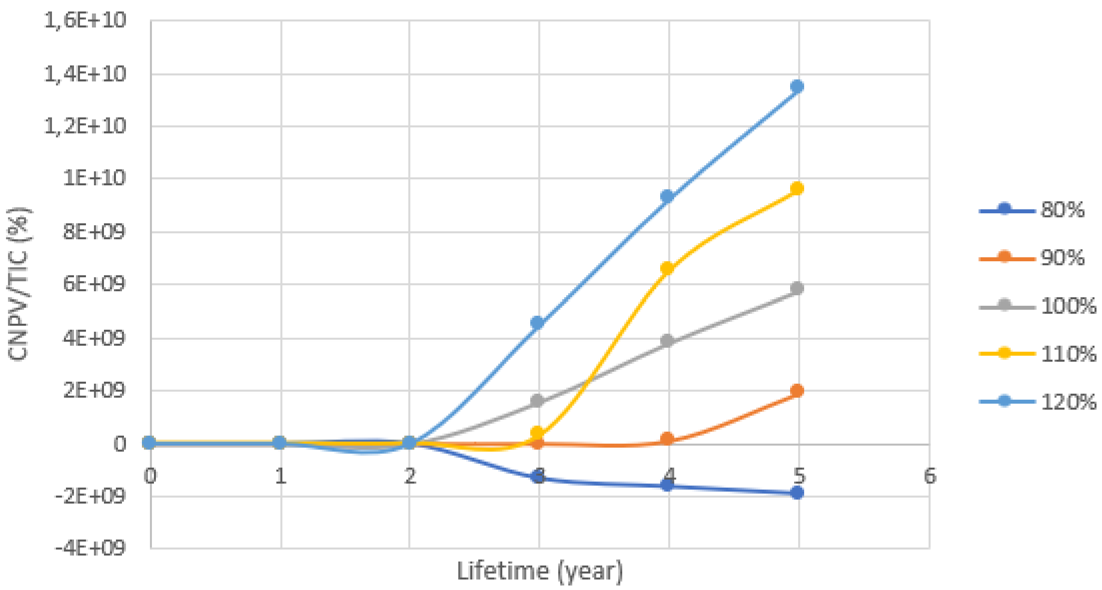


**Figure 5. CNPV curve of sales variation**

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### 3.2.4 Change in Variable Cost (Raw Materials, Utility and Labor)

Raw material, labor or utility can greatly affect the company. The greater the price of raw materials, the greater the capital required by the company. It's the same with labor and utilities. Many companies are looking for raw materials with the lowest value to get less capital, so the company will get bigger profits. **Figure 6** shows that the smaller the percentage of raw material variance, the greater the profit. However, of the five variants, all of them will experience a return on investment in the second year to the third year. In the 80% variant, the company will experience a return on investment in 2.2 years. But will continue to experience profits for years to come. Likewise with 90%, 100%, 110%, and 120%. However, in the 120% variant, the company will only get a lower profit than the other types of variance percentages. However, in the 120% variant, the company will only get a lower profit than the other types of variance percentages.

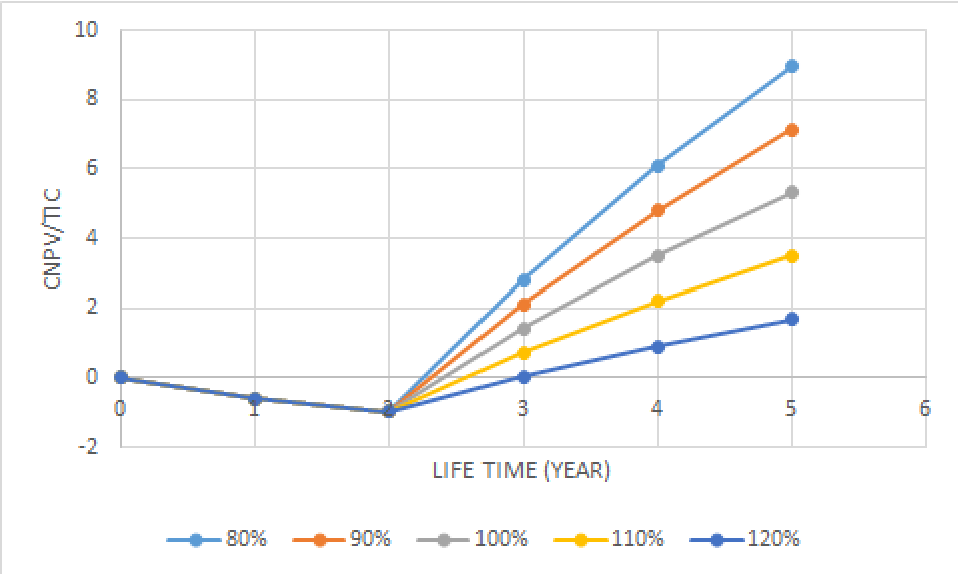


**Figure 6. CNPV/TIC with raw material price variations**

**Figure 7** shows that the higher the percentage of salary, the company will experience a longer return on investment. As happened in the 120% percentage variance, the return on investment occurred after the second year. However, from the variants 80%, 90%, 100% and 110% the return on investment will occur in the first year to the second year. Referring to the formed curve, there will be fluctuations in the company at a percentage of 120% which means the company's economy is running unstable. At a percentage of 80%, the company gets very high profits compared to the percentages of 90%, 100%, 110%.

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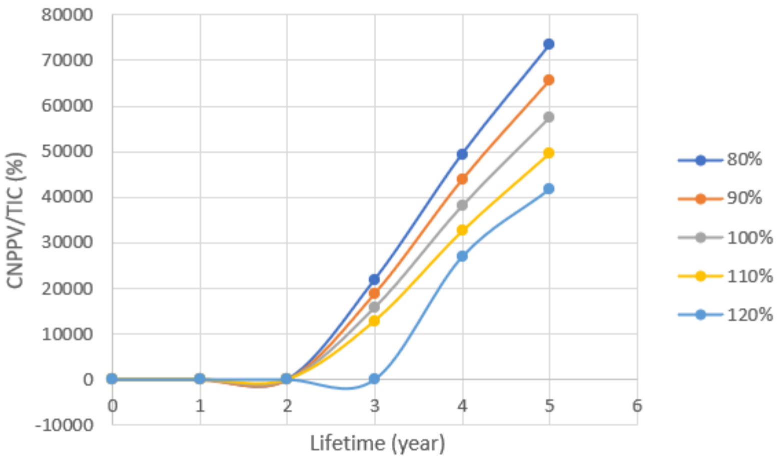


**Figure 7. CNPV/TIC with worker's salary variation**

**Figure 8** shows the CNPV/TIC graph is analyzed with various utility prices. The ideal utility price was 100%. The utility price variations used in this analysis were 80, 90, 100, 110, and 120%. The CNPV/TIC value from early to second year decreased slightly after the project was created. Then surprisingly, it increased from 2nd to 3rd year and so on. However, there was no significant effect of utility price variation on the CNPV/TIC graph. The project can still run and make a profit. The PBP results from the utility variations of 80, 90, 100, 110, and 120% will be achieved in 2,8 since the project was created.

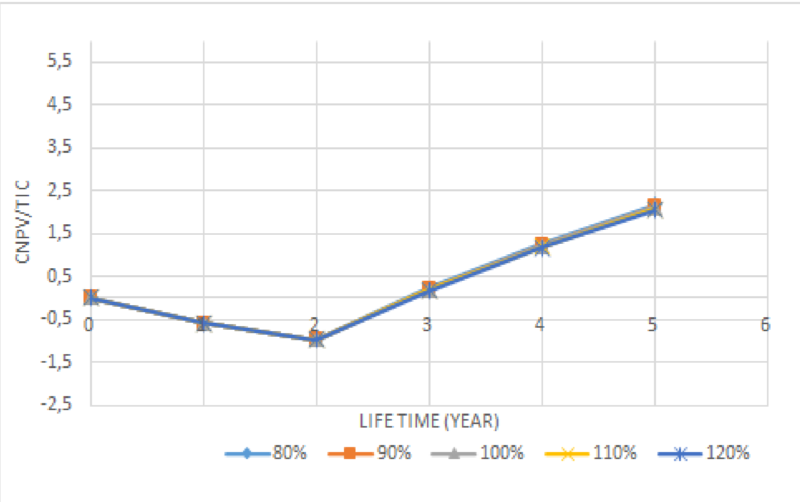


**Figure 8. CNPV/TIC with Utility variation**

## 4. CONCLUSION

Based on the above analysis, the production of hyaluronic acid by repeated batch fermentation using Streptococcus zooepidemicus is promising. Analysis by PBP shows that the investment is profitable after more than 5 years. This project can compete with PBP capital markets standards due to its short return on investment. Some of the things that influence this benefit include using the repeat batch method, as it is very simple, does not use hazardous materials, and is inexpensive. From these economic evaluation analyses, we can

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conclude that this project is feasible and the industry will become a promising project in the future.

## 5. AUTHORS' NOTE

We acknowledged Bangdos, Universitas Pendidikan Indonesia.

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