Preliminary Design of Phycocyanin Production from *Spirulina platensis* Using Anaerobically Digested Dairy Manure Wastewater

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Abstract. Phycocyanin is a blue pigment that is found in cyanobacteria such as *Spirulina platensis*. Its ability to provide stable and non-toxic blue color makes its market value is so high that it reaches USD 112.3 million in 2018. However, the cultivation of *S. platensis* to produce phycocyanin can only be carried out by large-scale industry, due to high operating costs of synthetic medium such as Zarrouk. We considered the use of Anaerobically Digested Dairy Manure Wastewater (ADDMW) as an alternative for growth medium to produce phycocyanin from *S. platensis*. A laboratory-scale study was conducted to determine the optimum composition of the medium containing ADDMW and NaHCO$_3$ as a carbon source for *S. platensis*. We found that the ADDMW medium combined with 16.8 g/L NaHCO$_3$ and 25 g/L NaCl resulted the highest productivity of phycocyanin among the other variations. Industrial-scale economic analysis of this integrated cultivation method showed its economic feasibility using 10 years lifetime analysis. Highest GPM (gross profit margin) obtained is 12.40 with IRR (internal rate of return) 23% which give 3.13 years of payback period. Further study is needed on technical aspect and actual implementation of industrial scale design.

1. Introduction

Phycocyanin is a blue pigment produced by blue-green algae (*cyanobacteria*) with an average content of 8-13% of dry algal biomass. It is utilized by cyanobacteria such as *Spirulina platensis* in the process of harvesting light through photosynthesis [1]. Its high antioxidant content [2] and stable color character make this pigment widely used in various industries so that it’s market value can reach USD 112.3 million in 2018 [3].

However, the production of phycocyanin from *Spirulina platensis* is still being constrained by high price of synthetic substrates. As a result, only large-scale industries are capable of producing phycocyanin, while smaller-scale industries can only sell the biomass in forms of powder or capsule [4]. To reduce the cost of raw materials, conventional substrates such as Zarrouk could be substituted using organic waste that still contains essential elements that can support the growth of *S. platensis*. In this preliminary design, the organic waste from Anaerobically Digested Dairy Manure Waste (ADDMW) which is the liquid waste of biodigester using cow manure was examined. ADDMW is chosen because of its abundant in Indonesian villages and still contains nitrogen and phosphorus compounds needed for *S. platensis* cultivation [5]. Combined with NaHCO$_3$ as a carbon source and NaCl to increase salinity, ADDMW could be a low-cost alternative medium for *S. platensis*. The lab-scale
experiments were carried out using NaCl and NaHCO3 variation to determine the optimum condition for the phycocyanin production from *S. platensis* using ADDMW as a substrate.

Gross Profit Margin analysis was performed to evaluate the profitability of laboratory-scale. The alternative process with the highest GPM value is then selected to be analyzed further using mass and energy balance. To evaluate the visibility of this preliminary design, an economic analysis was carried out using some calculations including Total Capital Investment, Total Production Cost, Internal Rate of Return (IRR) and Payback Period (PP). A sensitivity analysis with 50% and 150% variation on three main variables was also carried to assess the riskiness of this preliminary design.

2. Material and Methods

2.1. Spirulina Cultivation and Phycocyanin Analysis

Cultivation was conducted using 1 L culture bottle with a working volume of 800 mL. Aeration was given with a rate of 3 LPM, and light intensity of 4000 lux was used. Photoperiodism employed was 16 hours of light and 8 hours of dark. NaHCO3 and NaCl were varied for each system. NaHCO3 added is 0 g/L, 8.2 g/L and 16.4 g/L. Culture density was estimated using spectrophotometry through the optical density method. Nitrate and ammonium in the medium also quantified in this study using the APHA-4500-NO3-B-2012 standard and Nessler method.

Phycocyanin from Spirulina’s dry biomass was extracted using a cold maceration method at 4˚C temperature with phosphate buffer as a solvent. Maceration took place for 24 hours in dark chamber. The sample was then centrifuged at 5100 rpm for 2 minutes. The supernatant was measured using a spectrophotometer at λ 615 and 652 nm. Phycocyanin concentration was measured by equation (1).

\[
[\text{Phycocyanin}] (\mu\text{g/mL}) = \frac{A_{615} - 0.474A_{652}}{5.34}
\]  

(1)

2.2. Process Flow Diagram Analysis

The process flow diagram analysis used in this study is the Stage-Gate™ Product Development Process (SGPDP) [6]. This process used to develop several alternatives for phycocyanin production. Using an innovation map, this study distinguishes between each innovation in aspects such as raw materials, technical differentiation, products, and customer value. Also, as a preliminary study, Gross Profit Margin used to differentiate each innovation. Gross profit margin obtained by equation (2).

\[
\text{GPM} = \frac{\sum (Pp \times p) - \sum (Mc \times m)}{\sum (Mc \times m)}
\]  

(2)

Where *Pp* is the product cost, *p* is the amount of product produces, *Mc* is material cost and *m* is the material used to make an equivalent number of products. The selected option is developed into a process flow diagram using Superpro Designer® software.

2.3. Economic analysis method

To evaluate economic profitability, this study estimating the total capital cost by quotations from various sources and estimating the total production cost of phycocyanin powder. The cumulative cash flow of the plant with 10 years lifetime was also made with several assumptions. From the cumulative cash flow, net present value, payback period and internal rate of return were evaluated to give a projection of the design’s future economic condition in 10 years. To anticipate fluctuations that may happen in the plant’s lifetime, sensitivity analysis with three main variables, raw materials cost, employee salary, and production capacity, was made with a ±50% variation.

3. Results and Discussion

3.1. Lab-scale results

There is no significant difference between *S. platensis* cultivated in Zarrouk medium or modified ADDMW (Table 1). Phycocyanin concentration varied for every medium variation. Optimum
phycocyanin concentration obtained in ADDMW with the addition of 16.8 NaHCO$_3$ and 25 g/L NaCl. There is a slightly different phycocyanin concentration caused in higher NaHCO$_3$ concentration. HCO$_3^-$ have a significant role in $S$. platensis' photosystem II. Bicarbonate ion could replace glutamate as a ligand in non-Fe Heme [7], which could increase electron transport efficiency from QA to QB and production of ATP [8]. Previous study indicated that variation of NaHCO$_3$ has no significant impact on phycocyanin concentration in $S$. platensis [10]. However, the mechanism of bicarbonate ion increasing phycocyanin concentration remains unclear. On the other hand, salinity treatment in this study showed a slight significant impact on phycocyanin concentration. $S$. platensis is usually grown in high salinity water but less saline than seawater (35 ppt). In 35 ppt NaCl may cause disintegrated phycocyanobilin complex in the thylakoid membrane of $S$. platensis. In another study, the highest phycocyanin concentration obtained in 23 ppt NaCl [9].

### Table 1. Kinetic Parameter of $S$. platensis Cultivation

| Medium | $\mu$ (day$^{-1}$) | $dt$ (day) | Initial [NO$_3$] (g/L) | Initial [NH$_4$] (g/L) | Productivity (g L$^{-1}$ day$^{-1}$) | Phycocyanin (g/g biomass) |
|--------|-------------------|------------|------------------------|------------------------|------------------------------------|-------------------------|
| Zarrouk | 0.245             | 3.01       | 0.25                   | 0                      | 0.0468 ± 0.0017                    | 10.49%                  |
| ADDMW  | 0.228             | 3.05       | 0.02                   | 0.05                   | 0.0400 ± 0.0040                    | 1.91%                   |
| ADDMW + 16.8 g/L NaHCO$_3$ | 0.190 | 3.65       | 0.02                   | 0.05                   | 0.0607 ± 0.0010                    | 3.48%                   |
| ADDMW + 8.4 g/L NaHCO$_3$ | 0.205 | 3.43       | 0.02                   | 0.05                   | 0.0586 ± 0.0090                    | 0.77%                   |
| ADDMW + 16.8 g/L NaHCO$_3$ + 15 g/L NaCl | 0.207 | 3.49       | 0.02                   | 0.05                   | 0.0660 ± 0.0260                    | 2.25%$^a$               |
| ADDMW + 16.8 g/L NaHCO$_3$ + 25 g/L NaCl | 0.210 | 3.39       | 0.02                   | 0.05                   | 0.0800 ± 0.0130                    | 4.90%$^b$               |
| ADDMW + 16.8 g/L NaHCO$_3$ + 35 g/L NaCl | 0.232 | 3.00       | 0.02                   | 0.05                   | 0.0980 ± 0.0210                    | 2.30%$^a$               |
| Anaerobically Digested Pig Manure$^{10}$ | -     | -          | -                      | 200                    | 0.0670                             | 19.50%                  |

**3.2. Innovation map and GPM analysis**

An innovation map of phycocyanin production is made to compare the conventional method of using the Zarrouk medium and the integrated method of using the ADDMW medium (Figure 1). Both methods use a three-step pre-treatment process of sterilizing, freezing, and milling to optimize the extraction process [1]. In the conventional method, the Zarrouk medium is used and phycocyanin is produced from Spirulina biomass without any by-products made from the residue of the extraction process. Compared to the integrated method, where the ADDMW medium is used and fish feed is made from the biomass residue of the extraction process, the conventional method is costly and produces waste in the form of biomass residue. Biomass residue from the extraction process can be utilized further as fish feed, as it still has high protein content.
Based on the innovation map, the feasibility analysis of both methods for industrial-scale is made by designing a process for phycocyanin production with a production capacity of 5.93 ton/year. This production capacity is made to fulfill 0.46% of the world’s blue food coloring demand which reached 1,294 ton/year in 2017 [11]. The preliminary process design is based on the extraction method with a yield of 8.25% phycocyanin from Spirulina biomass [1].

Gross profit margin (GPM) analysis of these two methods showed that the integrated method is more profitable compared to the conventional method (Table 2 and 3). GPM of the integrated method reached 12.40 with a total revenue of USD 1,561,655.50 per year. Meanwhile, the total revenue of the conventional method is USD 1,542,840.00 per year with a higher production cost of USD 137,669.62 per year due to the high price of Zarrouk medium, resulting in a lower GPM value of 10.21 compared to the integrated method. The integrated method is seem to be more profitable than the conventional method.
3.3. **Process development**

Phycocyanin production starts with the cultivation of Spirulina in an open raceway pond system (Figure 2). The substrate used is ADDMW added with 1 kg/m$^3$ NaHCO$_3$ as an additional carbon source and 25 kg/m$^3$ of coarse salt (NaCl) to increase the salinity of the medium. The biomass produced from this cultivation process then enters the pre-treatment stage consisting of drying, sterilizing, freezing and grinding. This pretreatment process was carried out to reduce water content, to sterilize biomass, and disrupt biomass’ cell walls to increase the yield of phycocyanin in the extraction process [1]. In the next step, the extraction process is carried out by cold maceration method at 4˚C using water as a solvent. Phycocyanin extract obtained in the liquid fraction is dried using spray drying method to produce a phycocyanin powder. The biomass residue obtained from the extraction process is reprocessed into pellets as fish feed because it still has a high protein content. This production system preliminary design can produce phycocyanin powder as much as 5.93 tons/year and fish feed 67.97 tons/year.

### Table 2. Gross Profit Margin of Conventional Method

| Raw Materials       | Amount per Year | Unit | Price per Unit (USD) | Total Price per Year (USD) |
|---------------------|-----------------|------|----------------------|---------------------------|
| Zarrouk Medium      | 18,105          | m$^3$| 8                    | 137,385.05                |
| Maltodextrin        | 0.514           | Ton  | 554                  | 284.57                    |
| **Total Expense**   |                 |      |                      | 137,669.62                |

### Table 3. Gross Profit Margin of Integrated Model

| Raw Materials       | Amount per Year | Unit | Price per Unit (USD) | Total Price per Year (USD) |
|---------------------|-----------------|------|----------------------|---------------------------|
| ADDMW               | 18,105          | m$^3$| 2.77                 | 50,117.16                 |
| Crude Salt          | 1,548           | Ton  | 34.60                | 53,571.90                 |
| NaHCO$_3$           | 62              | Ton  | 203.30               | 12,590.39                 |
| Maltodextrin        | 0.514           | Ton  | 553.63               | 284.57                    |
| **Total Expense**   |                 |      |                      | 116,564.02                |

### Table 2. Gross Profit Margin of Conventional Method

| Product             | Amount per Year | Unit | Price per Unit (USD) | Total Revenue per Year (USD) |
|---------------------|-----------------|------|----------------------|-----------------------------|
| Phycocyanin Powder  | 5,934           | kg   | 260                  | 1,542,840.00                |
| **Total Revenue**   |                 |      |                      | 1,542,840.00                |
| **GPM**             |                 |      |                      | 10.21                       |

### Table 3. Gross Profit Margin of Integrated Model

| Product             | Amount per Year | Unit | Price per Unit (USD) | Total Revenue per Year (USD) |
|---------------------|-----------------|------|----------------------|-----------------------------|
| Fish Feed           | 68              | Ton  | 277                  | 18,815.50                   |
| Phycocyanin Powder  | 5,934           | kg   | 260                  | 1,542,840.00                |
| **Total Revenue**   |                 |      |                      | 1,561,655.50                |
| **GPM**             |                 |      |                      | 12.40                       |
3.4. Economic analysis

The economic performance of the preliminary design of *Spirulina platensis* cultivation using anaerobically digested dairy manure wastewater as a substrate to produce phycocyanin and fish feed is studied using several analyses. The total capital investment consists of fixed capital investment (FCI) and working capital investment (WCI). FCI consists of equipment and land costs and WCI was assumed to be 20% of the FCI. The estimation of the total capital investment of this design is USD 1,497,943.14 with a lifetime of 10 years.

Total production cost (TPC) consists of raw materials cost, utilities, employee’s salary, supervision (assumed to be 10% of employee salary) and equipment maintenance (assumed to be 1% of FCI). TPC of phycocyanin powder reached USD 85.77/kg phycocyanin. In order to gain enough profit to keep the plant’s maintenance and production cost, the sales price of phycocyanin used in this preliminary design is three times its production cost, USD 260/kg phycocyanin. This sales price is also lower than the average current market price of phycocyanin powder which reached USD 300/kg phycocyanin in 2016 [12].

The cumulative cash flow of the design was made in order to evaluate its economic profitability (Figure 3). The cumulative cash included a linear depreciation of 10% in 10 years lifetime with negligible salvage values, and corporate tax of 25% of gross profit based on Indonesian law (UU no 36 Tahun 2008 pasal 17(1) b tentang Pajak Penghasilan) resulting in a net profit of USD 775,463 per year. Capital investments were made at the beginning of the first year in order to multiply the Spirulina starter culture before the plant started operating. This means that the plant started to earn revenues from sales starting at the end of the first year.
In addition to the cumulative cashflow, several economic indicators were also calculated. This included NPV (net present value), payback period, and IRR (internal rate of return) (Table 4). NPV of the design is positive at the end of 10 years, reaching USD 2,240,603.38. The 10 years NPV is almost two times larger than its total capital investment with a payback period of 3.13 years. The minimum acceptable rate of return (MARR) used in this study is 18%, which is three times of the Bank of Indonesia 7-day (Reverse) Repo Rate. This preliminary design has IRR value higher than the MARR value which indicates its profitability and lower risk.

Table 4. Economic indicator of preliminary design of *Spirulina platensis* cultivation using anaerobically digested dairy manure wastewater as substrate to produce phycocyanin and fish feed

| Indicator       | Value         |
|-----------------|---------------|
| NPV             | USD 2,240,603.38 |
| Payback Period  | 3.13 years    |
| IRR             | 23%           |

This study designed a 10 years lifetime of plant operation, therefore fluctuation in the economy and labor market should be anticipated as it can pose a risk to the plant’s economic condition. A sensitivity analysis is used to anticipate this fluctuation risk on the total production cost of phycocyanin using three main variables i.e. raw materials, employee salary, and production capacity with a ±50% range (Figure 4). Based on the sensitivity analysis, a fluctuation in the raw materials cost and employee salary will not pose a huge risk on phycocyanin TPC. A 50% fluctuation on the raw materials will cause ±11.7% on phycocyanin TPC, while 50% fluctuation on the employee salary will cause ±24.4% on phycocyanin TPC. Meanwhile, a 50% decrease in the production capacity will cause a drastic 100% increase in phycocyanin TPC and a 50% increase in the production capacity will cause a 33% decrease in phycocyanin TPC. The production capacity of this plant must be kept stable throughout the plant’s lifetime as it can cause a drastic effect on the plant’s economic condition.
4. Conclusion
This study showed the feasibility of using ADDMW (Anaerobically Digested Dairy Manure Wastewater) as a cheaper alternative medium for phycocyanin production from *Spirulina platensis* in lab-scale. Industrial-scale economic analysis of this integrated cultivation method showed its economic feasibility with 10 years lifetime. Further study needed for detailed estimation of technical aspects and implementation of the actual industrial scale design.

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