

Single cell protein production of *Chlorella* sp. using food processing waste as a cultivation medium

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Abstract. The aim of this study was to investigate the effect of various food processing wastes on the production of single cell protein by *Chlorella* sp. Three various food processing wastes i.e. tofu waste, tempeh waste and cheese whey waste were used as cultivation medium for *Chlorella* sp. growth. Sea water was used as a control of cultivation medium. The addition of waste into cultivation medium was 10%, 20%, 30%, 40%, and 50%. The result showed that the highest yield of cell mass and protein content was found in 50% tofu waste cultivation medium was 47.8×10^6 cell/ml with protein content was 52.24%. The 50% tofu waste medium showed improved cell yield as nearly as 30% than tempeh waste medium. The yield of biomass and protein content when 30% tempeh waste was used as cultivation medium was 37.1×10^6 cell/ml and 52%, respectively. Thus, food processing waste especially tofu waste would be a promising candidate for cultivation medium for single cell production from *Chlorella* sp. Moreover, the utilization of waste can reduce environmental pollution and increase protein supply for food supplement or animal feed.

1. Introduction

Single cell protein (SCP) is an interesting object in research since early 1950. Single cell protein is the dried cells of microorganism, has been explored for being an alternative protein source as a food supplement or animal feeds as the preventive protein deficiency supply [1]. Some microorganism such as yeast, fungi, bacteria, and microalgae can be used in SCP production. This protein source is named as single cell protein because it describes the protein production from biomass (cell) that different from vegetable protein and animal protein. The production of SCP from various microbes gives some different characteristics either.

The production of SCP from fungi and bacteria has been received considerable attention, in contrast, only a few studies have dealt from SCP of microalgae. Comprehensive analysis and nutritional studies have demonstrated that these algae proteins are a high quality and comparable with conventional vegetable proteins [2].



Microalgae are the unicellular photosynthetic microorganism that grows in marine. Microalgae has diameter or length of approximately 3-10 μm . Microalgae metabolism reacts to change in the external environment with changes in its intracellular environment. Thus, the manipulation of the culture condition or the presence nutrients stimulates the biosynthesis of specific compounds [3]. Microalgae are the best choice for single cell protein productions because they also have simple and inexpensive growth requirements (free sea water, inexpensive nitrogen, phosphorus, and carbon sources) and rapid growth rates with sufficient light. Single cell protein production using microalgae has been known as an effective and economical way process. Microalgae are a potential source of protein. Cultivation of microalgae is the preferred way for the development and production of these high-value products [4].

Many studies have created to develop microalgae cultivation technique that purposes for decreasing production cost and produce a better quality of cell yield. Microorganisms like algae, fungi, yeast, and bacteria utilize inexpensive feedstock and wastes as sources of carbon, nitrogen and energy for growth to produce biomass and protein concentrate. A study shows that microalgae *Chlorella vulgaris* could grow well in tofu industrial waste [5]. It shows that nitrogen source in waste from food processing can be used as microalgae growth support.

Waste from food industry is one of causing the environmental pollution. Food industries such as tofu industry, tempeh industry and cheese industry are widely spread in Indonesia's areas. Waste from food industries contains complex organic material, such as carbohydrates, protein, lipids, and several microelements. The protein contained in its raw waste occupies about 60% of all solid fraction depends on the characteristic of the waste [5]. Protein can be degraded into various organic compounds, which some of them can be used either as macronutrient or micronutrient for the autotrophic organism, such as microalgae. It is expected that waste of food industry is able to enhance the microalgae cultivation. In this study, three various food processing wastes i.e. tofu waste, tempeh waste, and cheese whey waste were utilized as a nitrogen source for microalgae growth. Among various microalgae, *Chlorella* sp. was selected in this study as single cell producer because of its high growth rate, high protein content, high chlorophyll content and low nucleic acid content.

2. Materials and methods

2.1. Microalgae strain and cultivation medium

Chlorella sp. strains were obtained from Balai Budidaya Air Payau (BBAP) Situbondo, East Java, Indonesia. Tofu waste was obtained from Dusun Gendangan, Kabupaten Malang, tempeh waste was obtained from Sanan, Kota Malang, and cheese whey waste was taken from Pujon, Kabupaten Malang. The cultivation medium consisted of control medium and modified medium growth. Control medium was contained filtered seawater and Walne as nitrogen source. The modified medium contained seawater and each various waste with 10%, 20%, 30%, 40% and 50% concentration. Seawater was sterilized at 121°C for 15 min [6]. The tofu waste and tempeh were pasteurized at 70°C for 15 min [7]. The cheese whey waste was sterilized at 121°C for 15 min. Volume of medium growth was 300 ml. *Chlorella* sp. utilized waste as a nitrogen source such as nitrate during the growth. Table 1 shows the nitrate content of each nitrogen source.

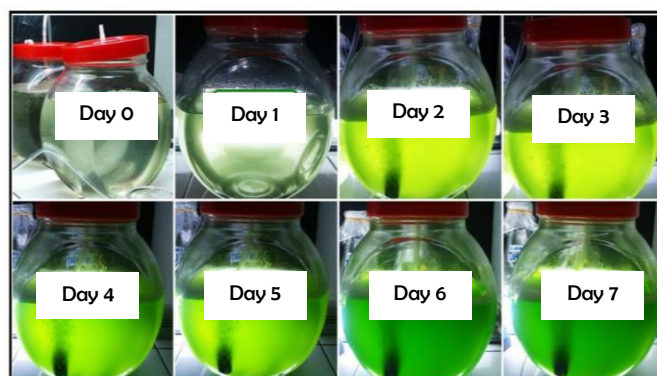
Table 1. Nitrate (NO_3) content.

Nitrogen Sources	Nitrate (ppm)
Tofu waste	9.21
Tempe waste	13.33
Cheese whey waste	14.70
Walne	1.9

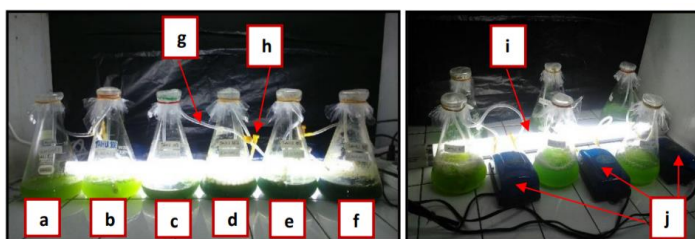
The initial pH of modified medium was adjusted from 3.5 to 7.0 using NaOH 1N solution. Vitamin B12 was added as much as 0.3 ml into each cultivation medium as a supported growth of microalgae. Microalgae *Chlorella* sp. added into medium was 30 ml.

2.2 Cultivation conditions

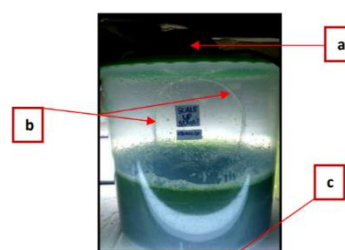
Chlorella sp. was grown in each modified medium at 25-35°C with aeration, salinity was adjusted at 30-34 ppt and pH was adjusted at 7.5-8.5[8]. Light source of 18-watt lamp (2500 lux) was used in this study to give the optimum growth of *Chlorella* sp. The proper Dissolved Oxygen (DO) in medium was 5-7 mg/l. Cultivation of *Chlorella* sp was did in three step which are to produce stock culture, determine the effect of each modified medium, then scale up (Figure 1).



(I)



(II)



(III)

Figure 1. (I) Stock culture of *Chlorella* sp; (II) Waste medium formation of *Chlorella* sp - (a) control medium (b) 10% waste medium (c) 20% waste medium (d) 30% waste medium (e) 40% waste medium (f) 50% waste medium (g) aeration tube (h) aeration regulator (i) 18-watt lamp (j) aerator ; (III) Scale up - (a) aerator (b) aeration tube (c) 18-watt lamp

2.3 Analysis

Aliquots of 300 ml were taken every 24 hours to determine cell concentration, temperature medium, pH medium, salinity, and Dissolve Oxygen (DO). Cell concentration was determined by microscope using haemocytometer method [6]. Temperatur and DO were determined by DO meter, salinity was determined by handrefractometer, pH was determined by pH meter [9]. Protein content was determined every 48 hours. The protein content of *Chlorella* sp. was determined by using Biuret method [10]. Nitrat content in medium growth was determined in day 0 and day 10 using spektrofotometry method.

3. Results and discussion

Chlorella sp. cultivation media in the study included control media and waste media. Waste media included tempeh waste, tofu waste, and cheese whey waste. Each waste medium was added 10%, 20%, 30%, 40% and 50% of waste. The more concentration of waste added to the growth medium, the higher the increase of the nitrate content of the growth media. The nitrate content of the growth media with various concentrations of waste is shown in Table 2.

3.1 Protein production by *Chlorella* sp.

Increased protein occurs due to the presence of nitrate assimilation by *Chlorella* sp. Assimilation of nitrate during the growth period produces glutamate. Glutamate is one of the protein amino acids [11]. Glutamate will undergo metabolism to produce other amino acids to form proteins. Figure 2 presents an increase of protein content from three types of waste.

Table 2. The Nitrate (NO₃) content of the growth media.

Media	Nitrate (ppm)
Control	1.90
Tofu waste medium 10%	0.92
Tofu waste medium 20%	1.84
Tofu waste medium 30%	2.77
Tofu waste medium 40%	3.68
Tofu waste medium 50%	4.61
Tempe waste medium 10%	1.33
Tempe waste medium 20%	2.66
Tempe waste medium 30%	3.99
Tempe waste medium 40%	5.32
Tempe waste medium 50%	6.65
Cheese waste medium 10%	1.40
Cheese waste medium 20%	2.81
Cheese waste medium 30%	4.22
Cheese waste medium 40%	5.63
Cheese waste medium 50%	7.04

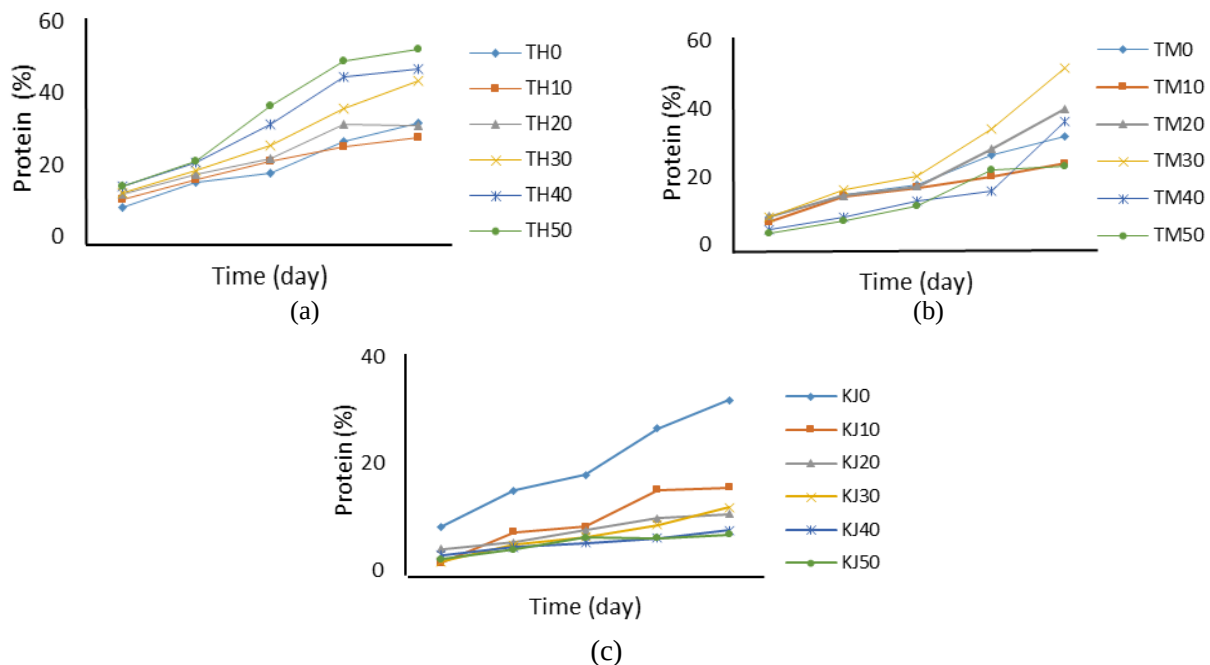


Figure 2. The effect of waste concentration on protein production (SCP) by *Chlorella* sp.

TH10 = Tofu waste 10%; TH20 = Tofu waste 20%; TH30 = Tofu waste 30%; TH40 = Tofu waste 40%; TH50 = Tofu waste 50%; TM10 = Tempeh waste 10%; TM20 = Tempeh waste 20%; TM30 = Tempeh waste 30%; TM40 = Tempeh waste 40%; TM50 = Tempeh waste 50%; KJ10 = Cheese whey waste 10%; KJ20 = Cheese whey waste 20%; KJ30 = Cheese waste 30%; KJ40 = Cheese whey waste 40%; KJ50 = Cheese whey waste 50%.

3.2 Effect of waste type as nitrogen source

Based on the results, growth media with the addition of different waste produced a different effect on the total protein of *Chlorella* sp. The highest total protein was found when 50% of tofu waste was added in the medium growth. Table 3 shows the protein content of *Chlorella* sp. in waste media and control media.

Table 3. Cell concentrate and total protein of *Chlorella* sp.

Medium	Cell concentrate (cells/ml)	Total Protein (%)
Control	$17,67 \times 10^6 \pm 2,25$	$31,80\% \pm 3,10$
50% of Tofu waste	$42,52 \times 10^6 \pm 2,80$	$52,32\% \pm 3,31$
30% of tempeh waste	$37,17 \times 10^6 \pm 1,52$	$52,00\% \pm 1,80$
10% of cheese whey waste	$8,5 \times 10^6 \pm 1,45$	$15,43\% \pm 2,55$

Table 3 shows the protein concentration of cell higher when waste media was used for protein production than that of control media. However, the protein concentration of *Chlorella* sp. in cheese whey waste media was lower than that of the control media. The main source of nutrition for the growth of *Chlorella* sp. is carbon, nitrogen, and phosphorus content [12]. The carbon source comes from CO₂ obtained from the atmosphere and organic carbon. Nitrogen sources are utilized in the formation of proteins in the form of ammonium and nitrate. The source of nitrate needed to support the growth of *Chlorella* sp. is 0.9-3.9 mg/l. Phosphorus is used as a universal energy source in the photosynthesis, respiration, and protein formation process; the ratio of nitrogen and phosphorus needs is 15:1 to 30:1 [12]. One of the nutrient contents in waste that causes an increased number of *Chlorella* sp. cells is nitrate [13]. The same nutrient content is found in tempeh waste that supports the growth of *Chlorella* sp. cells. This indicates that tofu and tempeh waste have the main nutrient source for the growth of *Chlorella* sp. [14]

Based on the results, the addition of 50% of tofu waste in growth media was able to support the increase of biomass, while the addition of 30% of tempeh waste in growth media could support the increase of cells. These differences are caused by the differences in waste characteristics that can affect the growth of *Chlorella* sp. These characteristics include nutritional content, clarity, and color of waste. However, the addition of cheese whey waste inhibited the growth of *Chlorella* sp. It assumed that the high nitrate content (14.07 mg/l) in cheese whey waste have the contribution for the growth inhibition. Moreover, it is suspected that the presence of lactose content in cheese whey waste also have plays a role in inhibiting the consumption of nitrate by *Chlorella* sp. The lactose content in cheese whey waste ranges from 5-6% [15]. Moreover, Garcia *et al.* [16] confirm that the source of lactose is not able to support the growth of microalgae. The source of carbon that can be used by microalgae is glucose. Glucose is a beneficial carbon source for microalgae as well as other species of microorganisms. The increased growth and respiration rates can be affected by carbon sources or glucose compared to other carbon sources, where glucose is used by microalgae to form other organic compounds such as lipids and proteins [17].

3.3 Nitrate utilization of *Chlorella* sp.

Based on the data, the nitrate content in the raw materials varies. The following is a table of nitrate content of culture media at the beginning and end of cell growth (Table 4).

Table 4. The Decrease in the Nitrate Content of the Growth Media.

Medium	Initial (ppm)	Final (ppm)	Consumption (%)
Control	1.9	0.47	75.26 ± 1.05
TH50	4.61	0.31	93.27 ± 0.78
TM30	3.99	0.48	87.97 ± 0.50
KJ10	1.40	0.52	62.62 ± 1.09

During the growth period, the nitrate content decreases. This is because nitrate is used by *Chlorella* sp. in the metabolic pathway. Based on the table, the nitrate on TH50 medium has been consumed more than the nitrate on TM30 and KJ10 media, with a percentage of 93.27%. This is because the growth of biomass in the TH50 medium is higher than in TM30 and KJ10 media. This is equivalent to the amount of protein produced, which is higher than the production of protein on TM30 and KJ10 media.

4. Conclusion

Tofu waste with a concentration of 50% produces 42.5×10^6 cells/m, with a total protein of 52.32%. Tempeh waste with a concentration of 30% produces 37.1×10^6 cells/ml, with a total protein of 52%. Cheese waste with a concentration of 10% produces 8.5×10^6 cells/ml, with a total protein of 15.43%. This study indicated that tofu waste has potential to be used as cultivation medium for SCP production.

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