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Production of Liquid Fertilizer from Chicken Feather Waste by Using Subcritical Water Treatment for Plant and Algal Growth

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Abstract. Chicken is one of the most popular protein sources for majority of the population, and million tons of chicken feathers waste are generated every year in Malaysia. However, it is a potential renewable source because feather is rich in keratin proteins and amino acids. In this study, the chicken feather was hydrolyzed into high value-added organic fertilizers by subcritical water (SCW) technology, and the effect of the SCW organic liquid fertilizer were investigated on the growth of spinach plant and red algae *Galdieria sulphuraria.* The resulted SCW liquid fertilizer contained 34,200 mg/L of nitrogen and 1,380 mg/L of phosphorus. The agronomic parameters of the spinach plants supplied with SCW liquid fertilizer and commercial fertilizer grew better compared to control plants. *G.sulphuraria* growth in 2x Allen's medium with addition of SCW liquid fertilizer, glucose and peptone, respectively was indicated by OD at 750 nm using a UV-VIS spectrophotometer. Thus, the utilization of SCW liquid fertilizer affecting the growth of spinach plants and *G.sulphuraria* are possibly eco-friendly approach in poultry waste treatments.

Keywords: Subcritical water, fertilizer, chicken feather, *Galdieria*

1. Introduction

Poultry is the world's primary source of animal protein which contribute to human nutrition with high quality protein and low level of fat. According to Food and Agriculture Organization of the United Nations, the demand for poultry meat production has increased rapidly from 9 to 120 million tons between 1961 and 2016 [1] and this is expected to continue in the future. The most abundant poultry wastes, feathers, constitute 10% of total chicken weight. The extensive poultry production has generated more than 7.7×10^8 tons/ year of feathers as a by-product of poultry industries [2], which can be disposed through landfill, combustion and composting [3]. These disposal methods lead to the environmental problems such as landfill, incineration, air and water pollutions, odor release, long residence time and pathogen production [4, 5]. In developed country, the feather wastes which disposed via steam and chemical treatments, not only result in low nutrition value of fertilizer but also consume large amount of energy and cost.

Subcritical water (SCW) treatment is proposed as a more eco-friendly option to hydrolyze organic feathers waste due to high temperature point (up to 374.2ºC) with respect to its properties such as density, dielectric constant, ion concentration, diffusivity and solubility [6]. Chicken feathers which is rich in amino acid and 90% of keratin protein [7,8], can be converted into value-added organic liquid fertilizer. Amino acid has specific benefits for improving the plant growth as it has been demonstrated to cause rapid release of nitrogen, N into the soil solution [9]. Indeed, the macronutrients such as nitrogen, phosphorus and potassium in the chicken feathers are essential for the plant growth [10].

In this study, the chemical analysis of SCW organic liquid fertilizer was performed to determine the total nitrogen (TN) and total phosphorus (TP). The efficiency of SCW organic liquid fertilizer was evaluated on the growth of spinach plant, it was compared with the commercial fertilizer. The spinach plants are easy to cultivate as they uptake low water consumption to be grown rapidly under bright sunlight with temperate temperature of Malaysia's environment [11]. The ability of SCW organic liquid fertilizer to support the cultivation of *Galdieria sulphuraria* was comparable to those of the commercial glucose and peptone. *G. sulphuraria* is metabolically flexible as it can be grown under photoautotrophic, heterotrophic, or mixotrophic conditions on more than 50 carbon sources [12,13]. Besides, *G. sulphuraria* also has ability to consume a wide variety of sugars and nitrogen sources. In algal cells, nitrogen is an essential constituent of all structural and functional proteins and accounts for 7%–20% of cell dry weight [14]. The cell structure of *G. sulphuraria* provides an advantage for the species to uptake minerals as energy and to survive in extreme acidic environments [12, 15].

2. Materials and methods

2.1 Chicken feathers hydrolysis subcritical water (SCW) treatment

The chicken feathers were collected from a broiler farm, Kedai Ayam Zahid in Banting, Selangor. The chicken feathers were dried under sunshine for seven days. Fifty grams of the dried chicken feathers mixed with 250 mL and 5 grams of $Ca(OH_2)$ were solubilized into organic liquid fertilizer by using the subcritical water (SCW) treatment in a batch reactor and fabricated by OM Labtech Co., Ltd. The operating condition were 180ºC; 100 rpm and 30 minutes for operating temperature, stirring speed and reaction time, respectively.

The obtained slurry products were vacuum filtered by using 0.45μm membrane filter paper to separate liquid products from solid residue. The solid was discharged and the liquid product was kept in a bottle as the SCW organic liquid fertilizer after adjusted to pH 6.0.

2.2 Determination of total nitrogen (TN) and total phosphorus (TP)

The concentration of SCW organic liquid fertilizer was compared to commercial liquid fertilizer based on two macronutrient contents analysis: total nitrogen (TN) as nitrate and total phosphorus (TP) as phosphate. TN and TP were analyzed by NitraVer5 Nitrate Reagent Powder Pillow system and PhosVer3 Phosphate Reagent Powder Pillow system, respectively, according the manufacturer's instruction (Hach, USA). Ten mL of the sample was mixed with 10 mL of NitraVer5 Nitrate Reagent Powder Pillow or PhosVer3 Phosphate Reagent Powder Pillow in a vial and shaken vigorously for 1 min. After 5 min reaction time, the samples were analyzed using HACH DR 6000; under the program 355 N and program 490 P React PP, respectively.

2.3 Effectiveness of SCW organic liquid fertilizer

The SCW organic liquid fertilizer was evaluated as potential fertilizer to see its effect on the growth of the spinach plant and cultivation of *G. sulphuraria.*

2.3.1 Growth of spinach plant

Spinach plants were grown as the test plants to evaluate their growth performance under different types of fertilizers application. In total, three fertilization treatments were conducted; SCW organic liquid fertilizer, commercial fertilizer (Baja AB Fertigasi, Pewaris Bumi Hijau, Malaysia) and no fertilizer as a negative control. Each triplicate of spinach plants in three different pots were supplied with 25 mL of liquid fertilizer once a week. The SCW organic liquid fertilizer was diluted 600 times with distilled water before applied to the spinach plants [16]. The commercial fertilizer was diluted 600 times. The spinach plantations were carried out at the rooftop of MJIIT building, Kuala Lumpur, in a pot (height: 14cm, width: 15cm and length: 15cm) and were watered daily. The growth parameters include the shoot height, leaf length, leaf width and number of leaves were measured after 30 days plantation. The data of each parameter were presented as the average in cm per plant (cm/plant).

2.3.2 Cultivation of G. sulphuraria

The algal strain *G. sulphuraria* 074 W (NIES-3638) was cultured in modified 2x Allen's medium [$(NH_4)_2SO_4$, 2.62 g/L; KH_2PO_4, 0.54 g/L; $MgSO_4$•H_2O, 0.50 g/L; $CaCl_2$•$2H_2O$, 0.28 g/L; $FeCl_3$•$6H_2O$, 16 mg/L; $MnCl_2$•$4H_2O$, 3.6 mg/L; $ZnCl_2$, 0.21 mg/L; $NaMoO_2$•$2H_2O$, 0.78 mg/L; $CoCl_2$•$6H_2O$, 80 µg/L; $CuCl_2$, 86 µg/L, pH 2.5 adjusted using H_2SO_4] at 40°C under the continuous illumination of a white fluorescent lump (ca. 100µ photons m-2 sec-1) with 24 hours aeration from the air pump [17]. Each modified 2x Allen's medium was separately added with different organic sources of nitrogen and carbon source to compare their growth (0-21 days). The cultures of *G. sulphuraria* were incubated in modified 2x Allen's medium with three organic sources: Bacto peptone (BD Biosciences, USA) as the peptone source, 4.5 g/L; SCW organic liquid fertilizer, 25 mL/L; and glucose, 4.5 g/L. For each condition, the growth of *G. sulphuraria* was determined by the turbidity at 750 nm using a UV-Vis spectrophotometer (UV-1900, Shimadzu, Japan) every 3 consecutive days.

2.4 Specific growth rate and statistical analysis

Specific growth rate (µ) was calculated using the two points of commencement of the exponential growth, indicated by the OD (Eq.1).

$$\mu = \ln(OD\,2/\,OD\,1)/(t\,2 - t\,1) \quad (Eq.1)$$

Significant differences in the OD of *G. sulphuraria* between two data points were tested by using T-test [two-tailed sample assuming unequal variances; statistical package, Excel (Microsoft)]. The results were considered significant if the p-values were ≤ 0.05.

3. Results and discussion

3.1 Production of SCW organic liquid fertilizer

The chicken feather wastes were hydrolyzed under SCW hydrothermal treatment process to produce the dark brown organic liquid fertilizer. The color changed indicated the degradation and chemical modification during the physical changes (data not shown). During subcritical liquid phase condition under SCW hydrothermal treatment, water molecules act as an acid-base catalyst which ionized into hydroxonium ion (H_3O^+) and hydroxide ion (OH^-) to enhance the solubility of treated organic chicken feather waste by maintaining pressure and temperature. Baskar et al. (2012) reported that the concentrations of H_3O^+ and OH^- ions are 100 times larger under subcritical water condition compared to water at ambient conditions [18]. Thus, the same ratio of mole of H_3O^+ and OH^- ions make the SCW organic liquid fertilizer become neutral rather than being acidic or basic.

3.2 Analysis of the concentration of total nitrogen (TN) and total phosphorus (TP)

Raw feathers are difficult to degrade by most proteolytic enzymes due to the presence of 90% keratin protein [19]. Feathers can hydrolyze the rich contents of protein and amino acid [20] to renewable source of good nitrogen fertilizer for plant nutrition at about 15% nitrogen content.

The analysis of the macronutrient concentration in the SCW organic liquid fertilizer showed that TN concentration (34,200 mg/L) and TP concentration (1,380 mg/L) were low than commercial fertilizer which are 250,000 mg/L and 232,000 mg/L for TN and TP, respectively (Table 1). However, TN concentration of SCW organic liquid fertilizer in this study was increased by 1,020 mg/L compared to the reported previously by Yoshikawa et al. (2015) [16]. This result indicated that SCW organic liquid fertilizer application gives a positive impact on the growth of plants for the next analysis. The TN and TP were 57 mg/L and 2.3 mg/L for SCW organic liquid fertilizer and 416.67 mg/L and 386.67 mg/L for commercial chemical fertilizer, respectively after dilution.

Table 1 Concentration of macronutrients in different samples.

Samples	TN concentration (as nitrate, mg/L)	TP concentration (as phosphate, mg/L)
SCW organic liquid fertilizer	34,200	1,380
Commercial chemical fertilizer	250,000	232,000

3.3 Effect of SCW organic liquid fertilizer on the growth of spinach plant

The effect of the fertilizers on the growth of spinach plants was summarized in Table 2. After 30 days plantation, the visual observation of spinach plants was observed larger and healthier with the addition of fertilizers compared to the control plant. The average value of shoot height (cm/plant), leaves length (cm/plant), leaves width (cm/plant) and number of leaves per plant was also increased significantly due to the application of fertilizers.

The average height of the spinach plants supplied with the different types of fertilizers was shown in Table 2. The highest shoot spinach plant (18.2 cm/plant) was recorded at commercial fertilizer application while the shortest (14.0cm/plant) was in control. Compared to the control plant, the length and width of the spinach leaves were increased with the addition of fertilizers application. The commercial fertilizer promoted the highest length and width of the spinach plant; (10.1 cm/plant) and (7.0 cm/plant), respectively. These results might be due to the rich nitrogen contents in the soil. The higher nitrogen contents in the fertilizer could improve the plant growth parameter.

The number of leaves in spinach plants increased with the addition of fertilizers in the soil. The maximum number of leaves per plant was counted in commercial fertilizer (12 leaves/plant) and significantly highest than any other pots (Table 2).

Table 2. Effect of fertilizers on shoot height, leaf length, leaf width and the number of leaves of spinach plant.

Source	**Spinach plant**			
Average parameter (cm)	**Height**	**Length**	**Width**	**Number of leaves**
No fertilizer	14.0	6.9	4.0	7
SCW organic liquid fertilizer	16.5	9.6	5.4	9
Commercial fertilizer	18.2	10.1	7.0	12

Thus, the spinach plants growth parameters were increased when SCW and commercial fertilizers were applied. However, the commercial fertilizers resulted in better performance for the growth of the spinach plants due to the higher amount of nitrogen and phosphorus contents as discussed in Table 1.

3.4 Effect of SCW organic liquid fertilizer on the cultivation of G. sulphuraria

Each cultures of *G. sulphuraria* in the 2x Allen's medium supplied with different carbon and nitrogen sources; glucose, SCW organic liquid fertilizer and peptone, respectively were incubated at temperature of 40ºC for 21 days. However, effects as carbon source would be more significant compared to that of the nitrogen source in this experiment because certain amount of $(NH_4)_2SO_4$ is involved in the medium. The extremophilic eukaryotic red algae *G. sulphuraria* can withstand temperature up to 56ºC [21]. The growth from day 3 to day 21 was used to evaluate the best performance of fertilizers on the growth rate of *G. sulphuraria.*

The visual images of *G. sulphuraria* cultured in the different carbon and nitrogen sources are shown in Figure 1. The light green color of the cultures in the modified 2x Allen's turns darker green with the addition of glucose and peptone. While *G. sulphuraria* cultivated in the modified 2x Allen's with the addition of SCW organic liquid fertilizer turns light brown color into brownish-green. This green culture means that *G. sulphuraria* starts to grow well in all nutrient conditions.

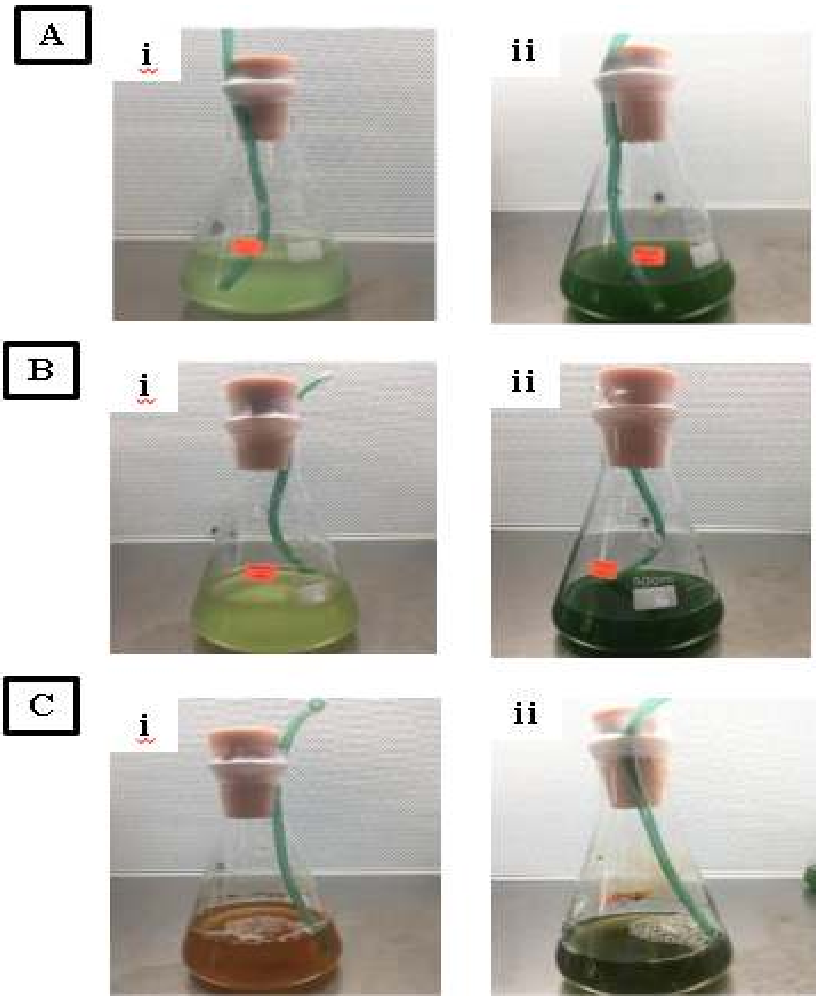

Figure 1. Culture of *G. sulphuraria* on (i) day 0 (ii) day 21. A: glucose, B: peptone, C: SCW organic liquid fertilizer.

In this study, *G. sulphuraria* was found to grow significantly faster in 2x Allen's medium with addition of SCW organic liquid fertilizer from day 0 to day 6 compared to the addition of glucose and peptone. In Figure 2, the growth curve of *G. sulphuraria* with the addition of SCW organic liquid fertilizer was higher than the addition of peptone had p-value of 0.803. Addition of commercial carbon and nitrogen source, peptone was used to elucidate its effectiveness on the growth of *G. sulphuraria*.

The growth from day 6 to day 9 was used to calculate the specific growth rates. The specific growth rate of *G. sulphuraria* with the addition of SCW organic liquid fertilizer (μ = 0.184 day^{-1}) was higher than addition of peptone (μ = 0.118 day^{-1}) conforming to the trends observed on the graph. Results indicate that the culture was able to obtain carbon and nitrogen sources more efficiently with addition of SCW organic liquid fertilizer than addition of peptone due to high composition of keratin protein, approximately 90% in the chicken feathers. In addition, the presence of carbon and nitrogen sources in algae cultivation would vary the trends of growth curve of algae.

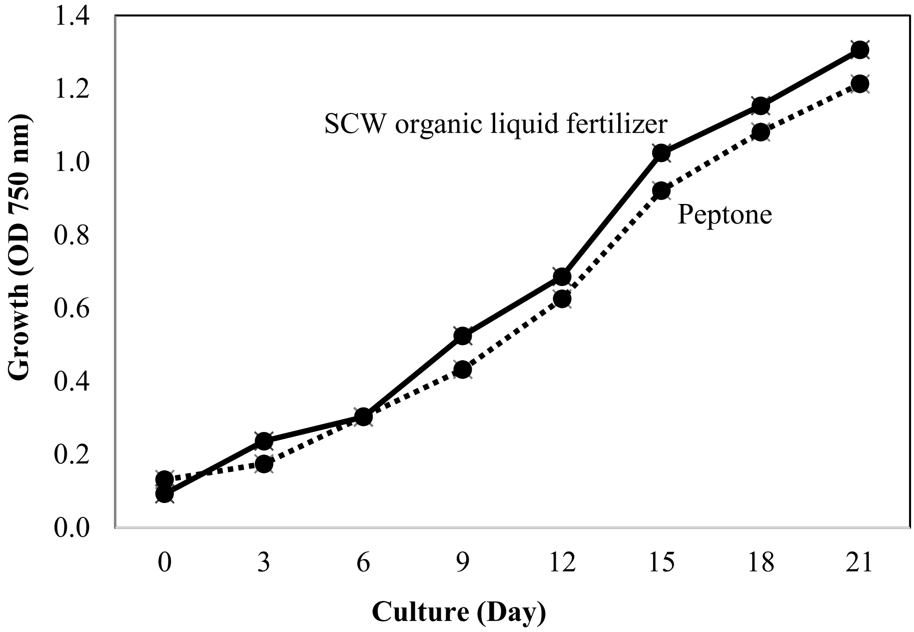

Figure 2. Growth curve of *G. sulphuraria* in 2x Allen's medium with addition of; solid line: SCW organic liquid fertilizer, dashed line: peptone.

Mixotrophic cultivation requires the large amount of synthetic carbon source (glucose) which act as the energy source for the growth of *G. sulphuraria.* In addition, mixotrophically cultivation of *G. sulphuraria* exhibited high growth rates compared with autotrophic or heterotrophic cells [22]. The growth of *G. sulphuraria* with addition of glucose was found to grow slightly better than addition of SCW organic liquid fertilizer (Figure 3). The specific growth rate of *G. sulphuraria* with addition of glucose (μ = 0.279 day^{-1}) was higher than addition of SCW organic liquid fertilizer (μ = 0.184 day^{-1}).

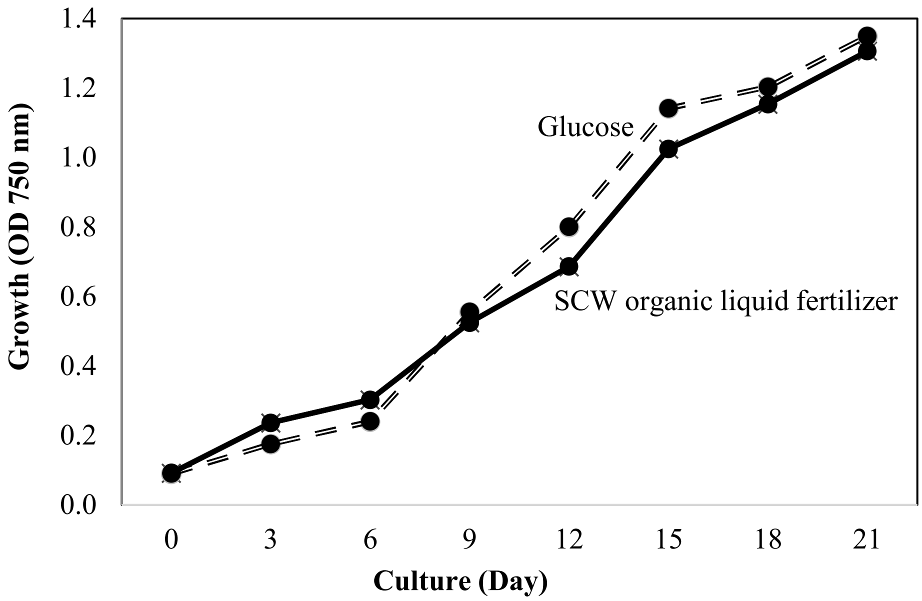

Figure 3. Growth curve of *G. sulphuraria* in 2x Allen's medium with addition of; solid line: SCW organic liquid fertilizer, double line: glucose.

4. Conclusion

Chicken feathers which considered as poultry waste could be a good SCW liquid fertilizer due to high content of keratin protein. The proper treatment via SCW treatment could promote an environmentally friendly approach. This SCW products could be utilized as liquid fertilizer source for the growth of plant and cultivation of *G. sulphuraria.*

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Figure legends

Figure 1. Culture of *G. sulphuraria* on day 0 and day 21. A: glucose, B: peptone, C: SCW organic liquid fertilizer.

Figure 2. Growth curve of *G. sulphuraria* in 2x Allen's medium with addition of: Diamond with solid line, SCW organic liquid fertilizer; diamond with dashed line, peptone.

Figure 3. Growth curve of *G. sulphuraria* in 2x Allen's medium with addition of: Diamond with solid line, SCW organic liquid fertilizer; diamond with double line, glucose.