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Production of *Spirulina sp* by Utilization of Wastewater from the Powder Type Energy Drinks

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Abstract. Wastewater of energy drink type of powder produced when the the production equipment required cleaning treatment to produce one taste to others. These equipments washed by water, so that, it produced wastewater. It contains high organic substances and classified as high degradable due to food product. The content of wastewater is high carbon and nitrogen substances. Microalgae is an autotrophic microorganism, live without carbon presence, utilized to digest the substances in wastewater especially for nitrogen substances. Spirulina sp is the type of microalgae selected to utilize the wastewater of energy drink, the selection criteria is the size of Spirulina sp is relatively large and easy to separated from its solution. The experiment conducted by cultivate the seeding microalgae with certain nutrients until the certain volume. The synthetic wastewater obtained from one of energy drink type of powder with commercial brand as Kuku Bima Ener-G, the wastewater concentration selected under the close to the real condition of wastewater as basis of COD measurement (6 sachet/L or COD of 12.480 mg/L) and aqueous concentration (1 sachet/L or COD of 2080 mg/L). The batch experiments with 1 L volume conducted and with variable of percent volume of wastewater added in order to observe the growth of microlagae. The response of the microalgae growth obtained by increasing the optical density of the microalgae solution and continued by calculation for the growth rate of microalgae. The result of the experiments indicated that for the aqueous concentration (1 sachet/L or COD of 2080 mg/L) the optimum added of wastewater is 40 % with growrate of 0.55/day while for the concentrated wastewater (6 sachet/L or COD of 12.480 mg/L), the optimum condition is 25 % wastewater added with growth rate of 0.43/day.

Keywords : energy drink, autotrophic, Spirulina sp, optical density, growth rate

INTRODUCTION

Efforts to search new sources of food and renewable energy have been conducted by researchers **[1] [2]**. This due to increase the world's human population, limiting source of fossil fuel and decrease the land to crop. One of the effort is to cultivate microalgal both as new source of food and new renewable energy. Microalgal has been studied since 1950s for those purposes **[3]**. This study especially focused as product as additive and food supplement and removal of certain material in wastewater **[4]**. *Spirulina sp* is one of type of microalgae has been intensively investigated by researchers to answer of those problem. *Spirulina sp* is a photosynthetic plankton, filamentous cyanobacterium with multicellular cylindrical trichomes in a helix along the filaments **[5]**. Indonesia as a tropical country has advantage to cultivate *Spirulina sp* because species can grows in tropical and subtropical country with pH of water body up to 11 **[6]**.

Powder type energy drink production discharged wastewater. The production of this type energy drink is solid-solid mixing. This wastewater produced by the cleaning of equipment from one taste to another taste product. Actually, this wastewater is a loss product because of solid-solid mixing process. The quality of wastewater from one product of the powder type energy drinks is pH : 4,16, COD : 12.350 mg/L, BOD : 9.320 mg/L **[7]**. This wastewater has high organic content as shown by its chemical oxygen demand (COD) and is also biodegradable. The main component of the energy drink is taurine (1000 mg/4-4,5 g) and then citric acid, sugar, and sodium bicarbonate. Taurine has function to digest lipid, to absorb vitamine dissolved in the lipid and to manage cholesterol in the body. For human body, taurine is not to give energy extra but to prevent skeletal muscles **[8]**. Effort to utilized this wastewater is started by separating the wastewater from other wastewaters with objective to maintain other compounds mixed in this wastewater. Separation of the wastewater is to set up condition due to algal species grow in selective media. Cultivation of *Spirulina sp* can be conducted in open-air

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cultures and still remain relatively free from contaminant by other microorganism **[3]**. Factors affecting the growth of *Spirulina sp* are pH, light intensity and presence of contaminants, nitrogen source, initial biomass concentration and population density **[5] [9]**. The high of COD content showed that this wastewater contains high organic compounds that can be further utilized. The component in this wastewater is a food product, it is also predicted useful to cultivate of *Spirulina sp* because source of carbon, nitrogen, phosphate, etc.

Microalgae is an autotrophic microbial and utilized N and C from CO_2 gas for cultivation **[10] [11] [12] [13]**:

$$11{,}4\ CO_2 + 2\ NH_4^+ + 6{,}8\ H_2O \rightarrow 2\ C_{5,7}H_{9,8}O_{2,3}N\ (\text{microalgae}) + O_2 + 2\ H^+$$

Through the fotosynthetic process, CO_2 from the aerob bacterial used by microalgae and also availability ammonia to form cell protoplasm and release oxgen molecule with light presence.

$$NH_3 + 8\ CO_2 + 4{,}5\ H_2O \rightarrow C_5H_{14}O_3N(\text{microalgae}) + 8{,}75\ O_2$$

Microalgal is a photosynthetic microorganisme and has potential use to product fine chemical **[14] [15]**, supplement food for human and animal **[16]**, immobilization system for generating extracellular compounds **[17]**, heavy metal biosorption **[18]**, and fixation of CO_2 **[19]**. Oil content of microalgal reachs 77 %, microalgal is also source of biodiesel, a new alternative renewable energy, and according to the calculation microalgal has ability to produce oil 200 times greater than other vegetable sources **[20]**. Environmental affecting the growth of microalgal is light intensity, water body temperature, pH, macro and micro nutrients, and CO_2 concentration **[21]**.

Recently, microalgal has also been exploited in the bioenergy field due to high content of carbon source. Type of microalgal for this experiment is *Spirulina sp* because it is easy to obtain surrounding Semarang City. This microalgal contains vitamines and the only one of microalgal contains vitamine of B complex (B1, B2, B3, B6, and B12 and minerals. The growth of microalgal observed by the density optic (OD) of microalgal using a double beam UV vis spectrophotometer (Shimadzu) and also calibration curve of OD and dry weight (g/L) of *Spirulina* sp biomass **[22] [5]**.

Wastewater of cleaning equipment, basically, is a product loss due to equipment required cleaning treatment to produce one taste to other products. Furthermore, the wastewater for experiment is a synthetic wastewater by dilution of product to a certain volume of distilled water. The sample of powder type energy drink is Kuku Bima Ener-G (KKB) with 4.5 g weight and has composition : extract of ginseng powder (300 mg), royal jelly (30 mg), honey (100 mg), taurine (1000 mg), caffeine (50 mg), vit B3 (20 mg), vit B6 (5 mg), vit B12 (5 mg), aspartam, cyclamat, acesulfame, sodium bikarbonat, citric acid, flavor, carmoisine CL 14720, dan dyes.

This research is pointed out to utilize of wastewater of cleaning treatment of equipment. This wastewater is basically degradable materials because of a food products. The real wastewater of energy drink type of powder from KKB product has characteristic of pH : 4,16, COD : 12.350 mg/L, BOD : 9.320 mg/L (Anonim, 2010). Based on the COD-value, this wastewater COD is equal to 6 to 7 sachet of KKB dissolved in the 1 L distilled water (1 sachet per litre of distilled water contains 2080 mg COD/L). Microalgae used in the experiments was *Spirulina sp,* it has advantages than other microalgae such as: the size is greater, easy to cultivate in tropical countries, and separated from the media than *Chlamydomonas sp*.

RESEARCH METHOD

Preparation of microalgae culture

Spirulina sp and Chlamydomonas sp. types of microalgae are cultivated by Integrated Laboratory of Diponegoro University, Semarang. Microalgae culture of *Spirulina sp* obtained from Integrated Laboratory of Diponegoro University, Semarang. These microalgae placed in the 200 mL of Erlenmeyer flask. Culture was inoculated with the addition nutrients. It consists of urea (0.05 g/L), trisuperphosphate or TSP (0.02 g/L), sodium bicarbonate (1 g/L), B-12 vitamin from IBI Pharmacy (0.5 tablet), sodium chloride (1.5 g/L) and sodium hydroxide to control the pH of 8-9 **[23]** and also sufficient aeration introduced to prevent precipitation of microalgae. This cultivation process conducted for five days **[24]**. Based on the color of microalgae that indicated the concentration of microalgae (2500 mg/L), it developed in to the 2 L reactor. The cultivation continued to the 100 L reactor until the concentration of microalgae sufficient based on the color of microalgae solution to be used for experiment.

Wastewater

Wastewater for the experiment was synthetic wastewater. This obtained by dissolved a powder type energy drink (Kuku Bima Ener-G, KKB) in the certain volume by distilled water. 1 sachet of KKB is 4.5 g weight (1

sachet of KKB dissolved in the 1 L distilled water has COD 2080 mg/L) **[25]**. The real wastewater of this is 6 sachets per distilled water.

Variables

Control variables conducted in the experiments are rate of aeration (1 l/min), salinity (1.5 gr/L), volume experiment (1 L), pH (8-9). While the independent variable is percentage volume and concentration of the wastewater volume in the culture and the response oberved is OD at 590 nm.
Fill the flask with culture microalga and wastewater as until 1 L volume, add the nutrients with composition mentioned above, set up the pH to 8-9 by sodium hydroxide. Turn on the 70 W of light **[26]** and aerator as a stirrer to prevent *Spirulina sp* precipitation in the bottom of the flask. Initial experiment measured the optical density and continuously observed the optical density every day until five days..

Biomass concentration analysis

Samples were taken daily and the optical density (OD) values were measured at 560 nm with a UV vis spectrophotometer (Shimadzu). The biomass concentration was calculated based on a calibration curve relating with the OD at 560 nm obtained, the values of biomass (dry basis) weight concentration was calculated according the formula : biomass (g/L) = 0.8236.OD560nm + 0.023 **[1] [27]**. This biomass concentration was obtained by weighing cell of microalgae after twice washing with distilled water and then drying in an oven at 80^0C during overninght until achieved a constant weight.

RESULT AND DISCUSSION

Production of *Spirulina sp*

The experiments were conducted in two type of wastewater, low and high wastewater. Low wastewater contained 1 sachet/L of powder type of energy drink and high wastewater is equal to 6 sachet/L distilled water based on the COD content. Converting COD into organic substance is equal to 2 % and 12 %. The total volume of the experiments were 1 L and variables conducted in the research is the percentage of the synthetic wastewater into microalgae culture. The result of the experiments shown in Figure 1 and 2.

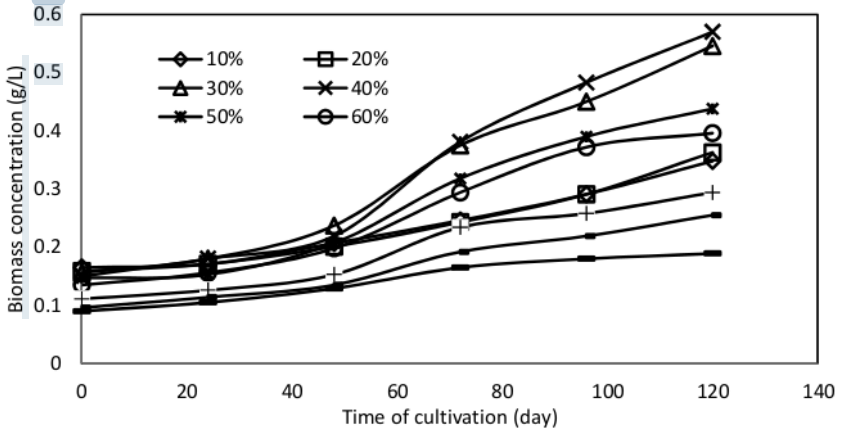

Figure 1. Low concentration of wastewaters to the of growth of *Spirulina sp*

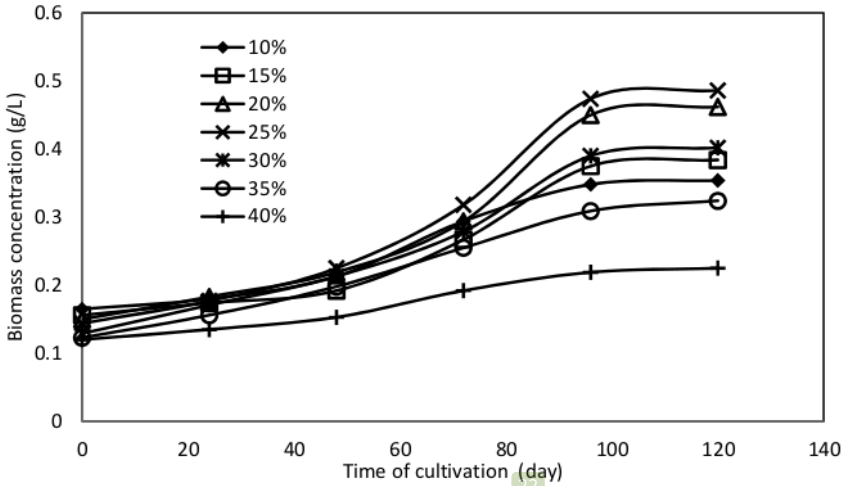

Figure 2. High concentration of wastewaters to the growth of *Spirulina sp*

The time of cultivation was limited of 120 hours with the consideration of the wastewater is fastly degraded. Microalgae can be grown under photoautotrophic, heterotrophic, and mixotrophic conditions using diversified carbon sources, such as carbon dioxide, methanol, acetate, glucose, other organic compounds **[28]**. Effects of cultivation time on the production of biomass, some researchers reported that this species had no lag phase **[22] [29]**. Increasing cultivation time increased biomass production. The results given in Figure 1 and 2 found that there were no lag phase of *Spirulina sp*. Figure 1 shown that the stationer phase until 30 % addition of waste water could not reach yet while the addition above 30 % the trend stationer phase closely reached. While Figure 2 indicated that the stationary phase achieved at the culture time of more than 4 days.

The main components of this energy drink is taurine and panax radix extract. Taurine is an amino acid derivat and panax radix extract is a natural phenolic compound. Both of these main components are the main nutrients of the growth of *Spirulina sp.*, At the low wastewater, the utilization of compounds in the wastewater by microalgae, until five days observation indicated that all portions of wastewater added into microalgae resulted similar trend of microalgae growth. At the portion of 40 and 30 % resulted the highest growth of the microalgae eventhough the stationary phase could not achieved yet. These portions of wastewater to be the best portion of wastewater and microalgae, microalgae able to utilize the nutrients to growth and can metabolise the organic carbon substrate **[30]** and also amino acid as source of nitrogen **[31]**. Above the 40 % of volume wastewater added, the growth of *Spirulina sp* tends to be decline, increasing the content of wastewater affected that compounds of wastewater especially taurine compound suppressed the growth of *Spirulina sp.* Exceess of taurine to the could result the physiological effect **[27]**. It could be understand that why the energy drink is only required in small portion because small portion would give positive effect but for higher portion would give decline effect to the microlagae growth. Generally, all portion of wastewater added to the *Spirulina sp* media until five days observation indicated that the stationer phase would not be reached, the growth of *Spirulina sp* is still increasing.

For high concentration of wastewater, the objective is to observe the ability of the *Spirulina sp* to utilize the nutritious components in wastewater, it could be seen from the Figure 2 that until four days obeservation the stationer phase have been achieved and the fifth days observation indicated that the *Spirulina sp* growth was stationer. The greatest growth for this condition is at the concentration of 25 % volume of wastewater. The growth of *Spirulina sp* in the high content of organic media indicated that the growth rate was lower than the low wastewater. Carbon sources are usually the most critical factors for the growth of microalgae. In general, microalgae can be grown under photoautotrophic, heterotrophic, and mixotrophic conditions using diversified carbon sources, such as carbon dioxide, methanol, acetate, glucose, other organic compounds **[28]**. Inhibition of growth of the *Spirulina sp* was due to excess of ammoniacal nitrogen **[32] [33]**.

Increasing the concentration wastewater could not increase the biomass of *Spirulina sp*, the components of wastewater are mostly in the form of organic compounds, while the type of this microalgae preferentially use inorganic nitrogen for growth, particularly nitrates and ammonium **[34] [35]**. The productivity of *Spirulina sp* by

utilization of wastewater of powder type energy drink was lower than using vinasse **[36] [37]**, phosphate concentrations **[5]**, ammonium sulphate and urea **[32]**.

Growth rate

The growth rate of *Spirulina sp* during cultivation calculated the equation by Fogg and Thake **[38]** as :

$$\mu = \frac{1}{t_2 - t_1} \ln \frac{OD_2}{OD_1}$$

where t = cultivation time and OD : optical density

The results of the growth rate were showed in the Table 1 and 2. For the low wastewater concentrations (1 sachet of powder type of energy drink) indicated that until the volume of 40 % (v/v) added, the growth rate increased with the maximum value of 0.5537 day-1. While increasing volume of wastewater the value of growth rate tend to decreased. At the low concentration of wastewater the value of has not reached the stationary phase, it could be higher of growth rate if the cultivation time increased.

Table 1. Growth rate of *Spirulina sp* at 1 sachet powder type energy drink

Time (1/day)	10% v/v	20% v/v	30% v/v	40% v/v	50% v/v	60% v/v	70% v/v	80% v/v	90% v/v
0-1	0.0357	0.0728	0.1431	0.1823	0.0400	0.1446	0.1268	0.1719	0.1542
1-2	0.1911	0.1616	0.2751	0.1961	0.3023	0.2384	0.1942	0.1691	0.2059
2-3	0.1726	0.1898	0.4589	**0.5537**	0.4293	0.3953	0.4249	0.3522	0.2461
3-4	0.1680	0.1803	0.1823	0.2372	0.2041	0.2353	0.0976	0.1316	0.0870
4-5	0.1789	0.2211	0.1934	0.1656	0.1161	0.0625	0.1306	0.1522	0.0488

Table 2. Growth rate of *Spirulina sp* at 6 sachet powder type energy drink

Time (1/day)	10% v/v	15% v/v	20% v/v	25% v/v	30% v/v	35% v/v	40% v/v
0-1	0.0870	0.1092	0.1989	0.2063	0.2819	0.2377	0.1178
1-2	0.1683	0.0984	0.1796	0.2400	0.2196	0.2384	0.1252
2-3	0.3223	0.3298	0.2945	0.3460	0.2699	0.2530	0.2271
3-4	0.1686	0.3397	**0.4257**	0.3992	0.3349	0.1921	0.1316
4-5	0.0171	0.0237	0.0263	0.0250	0.0303	0.0474	0.0270

The value of growth rate is closely to the result of previous result for cultivation of *Spirulina sp* **[5] [30] [39] [22]**.

The results indicated that *Spirulina sp* could more efficient utilize low concentration of wastewater compare as high concentration of wastewater. The high value of specific growth rate (μ) for low concentration of wastewater comfirmed that the metabolic efficiency of the alga was best on low concentration of wastewater **[34]**. The higher growth rate in low concentration may be due to greater efficiency of low concentration grown cells in the conversion of photosynthetically generated reducing power into net growth.

CONCLUSION

Utilization of wastewater of powder type energy drink has advantage in low concentration of wastewater, the best cultivation time of *Spirulina sp* was reached in addition of 40 % v/v with biomass product of 0,483 g/L and specific growth rate of 0.5537 h^{-1}. Increasing concentration of wastewater reduced the metabolic efficiency of alga. Eventhough, both the biomass product and specific growth rate was reaktively lower than previous studies but it is one of effort to utilize the wastewater rather than treated in wastewater treatment plant.

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