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Lipid production from tapioca wastewater by culture of *Scenedesmus sp*. with simultaneous BOD, COD and nitrogen removal

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Lipid production from tapioca wastewater by culture of *Scenedesmus sp.* with simultaneous BOD, COD and nitrogen removal

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Abstract. The use of microalgae to produce biodiesel or possibly remove nutrients from industrial wastewater has gained important attention during recent years due to their photosynthetic rate and its versatile nature to grow in various wastewater systems. In this study, a microalgae, *Scenedesmus sp.*, was cultured to enhance the lipid production and nutrients removal from tapioca wastewater sample. To assess lipid production, *Scenedesmus sp.* was cultured in different concentration of tapioca wastewater sample (from 0 to 100 %), and nutrient removal including BOD, COD, NH_4, NO_2, NO_3 level by *Scenedesmus sp.* was assessed in 100% of tapioca wastewater culture. After 8 days of culture, it was found out that 50% of tapioca wastewater sample resulted in highest concentration of lipid content than that of the other concentrations. The level of environment indicator as nutrient removal such as BOD, COD, NH_4, NO_2, NO_3 were also decreased up to 74%, 72%, 95%, 91%, and 91%, respectively. The pH condition changed from initial condition acidic (pH: 4) to neutral or basic condition (pH: 7-8) as recommended in wastewater treatment system. This research provided a novel approach and achieved efficient simultaneous lipid production and nutrients removal from tapioca wastewater sample by Scenedesmus's culture system.

Keyword: *Scenedesmus sp., tapioca wastewater, lipid production*

1. Introduction

The energy crisis is one of the most important problems faced by all people over the world in the 21st century. The highest consumption of fossil fuels has result in greenhouse effect and causes global climate change [1,2]. One of a type of renewable energy is microalgae biomass-based biofuel, which is considered as one of the most potent substitutes for fossil fuel [2]. However, to increase the production of microalgae biomass, several strategies should be developed, such as modification of culture medium and environmental factors. Hence, one of promising strategies is using wastewater sample as medium to enhance the algae biomass. The use of this wastewater could solve two environmental problems, (1) increase the lipid production of microalgae [1–3] and (2) decontaminate water pollution caused by wastewater treatments [3,4].

Indonesia is the 4th largest producers of cassava after Nigeria, Brazil and Thailand [5]. Due to the largest producer of this cassava production, many small or big factories working on cassava and produce many wastewater. Cassava processing wastewater produces several substances such as carbohydrates, nitrate, proteins, phosphate, potassium and cyanide that is highly polluting and must be treated before directing it to a water body [4–7]. Since several studies report the successfully cultivated microalgae in wastewater treatment [4,8,9], this study focused on using tapioca wastewater as the medium to enhance the lipid production for biodiesel-producing microalgae, *Scenedesmus sp.* In addition, *Scenedesmus sp*. was also assessed to decontaminate BOD, COD and nitrogen content in tapioca waste water sample. The result obtained from this study could be used as a protocol for lipid production and decontaminating tapioca wastewater substances.

2. Materials and methods

Microalgae *Scenedesmus sp.* used in this study was purchased from Surfactant and Bioenergy Research Center (SBRC) LPPM Institut Pertanian Bogor. This microalgae strain was maintained both on agar plates containing Bold's Basal (BB) medium and in liquid medium of Tauge Medium Extract (TME). The procedure to make TME medium referred to the protocol as described in detail by Prihantini [10].

2.1 Water qualities of tapioca wastewater

The tapioca waste water obtained from wastewater treatment plant of PT Naga Mas Sakti, Slorok Village, Kromengan Sub-district, Malang City, East Java, Indonesia was used as the culture medium in this study. In order to cultivate microalgal, the tapioca wastewater was filtered through filter paper to remove suspended solids and then stored at 4 ºC. The water quality of the tapioca wastewater sample is shown in Table 1.

2.2 Experimental Method

Scenedesmus sp. were cultivated in 100 mL of filtered tapioca wastewater sample using 250 mL flask. Tapioca wastewater sample was diluted into several concentrations of 5%, 10%, 20%, 30%, 40%, 50%, 75%, and 100%, to analyze their effect on the growth and lipid production of *Scenedesmus sp*. For inocula, 10 mL culture of pre-cultivated *Scenedesmus sp.* was centrifuged at 10,000 rpm, 5 min and 4°C. The microalgal pellets were washed twice with 30 mL of 20 mM HEPES solution. The inocula were then re-suspended in 5 mL of 20 mM HEPES solution and inoculated into each treatment. The initial microalgal density was approximately 10^4 cells per mL. The cultivation conditions were set as follows: light intensity: 5000 lux; light/dark ratio: 14 h:10 h, and temperature: 25 ºC. The cultivation was performed in triplicate (n = 3). The removal of biological oxygen demand (BOD), chemical oxygen demand (COD), NH_4, NO_3 and NO_2 as well as pH was assessed at 100% tapioca wastewater culture and the protocol referred to the standard protocols of American Public Health Association [11].

2.3 Analytical methods

The density of Microalgal cell were determined by cell counting using Neubour haemocytometer and assessed every day. At the end of the experiment all the culture was centrifuged and filtered using 0.45 µm membrane. Furthermore, dry weights of pellets were measured (80°C for 3 hours) to analyze the increase in biomass, cell count and lipid content. The lipid content was extracted from microalgal biomass using a modified method of Bligh and Dyer [12]. The lipids were extracted with chloroform–methanol (2:1, v/v), and followed by separation into chloroform and aqueous methanol layers through addition of methanol and water to generate final solvent ratio of chloroform: methanol: water of 1:1:0.9. The chloroform layer was washed with 20 ml of a 5% NaCl solution, and evaporated. Finally, the total lipids were measured gravimetrically.

2.4 Statistical analysis
All experiments were conducted in triplicate, and the averages of each treatment were reported with their standard error (SE). A one-way ANOVA analysis was used to evaluate the effects of different concentration of tapioca wastewater on the growth profile and lipid production of *Scenedesmus sp.* Furthermore, the significance of any differences was tested using Fisher's least significant difference (LSD) test.

3. Results and discussion

3.1. Water qualities of tapioca wastewater
In this study, water qualities of tapioca wastewater sample were determined in order to understand the baseline of water quality for Scenedesmus's culture. Based on Table 1, tapioca wastewater used in this study contains high concentration (mg/L) of $N\text{-}NO_3$ and COD, followed by $N\text{-}NO_3$, BOD, $N\text{-}NO_2$, N-NH4, K_2O and P_2O_5 respectively. Whereas, it contains low concentration (%) of solid residue of combustion and protein.

Table 1. Water qualities of tapioca wastewater sample used in this study

Index	Value
Protein (%)	0.33
Phosphorus (P_2O_5)(mg/L)	12.75
Potassium Oxide (K_2O) (mg/L)	39.97
Amilum (%)	3.92
Solid Residue of Combustion (Ash) (%)	0.02
Total Soluble of Biochemical Oxygen Demand (BOD) (mg/L)	245.09
Total Soluble of Chemical Oxygen Demand (COD) (mg/L)	499.23
Ammonium as Nitrogen ($N\text{-}NH_4$) (mg/L)	34.84
Nitrate as Nitrogen ($N\text{-}NO_3$) (mg/L)	269.94
Nitrite as Nitrogen ($N\text{-}NO_2$) (mg/L)	85.77
pH	4 – 4.5

3.2. The growth profile of Scenedesmus sp. in tapioca wastewater sample
The growth profile of *Scenedesmus sp.* was characterized under different concentration of tapioca wastewater (10%, 20%, 30%, 40%, 50%, 75%, and 100%) and culture temperature of 25 ºC. A one way ANOVA analysis revealed that there is significant different of the growth of *Scenedesmus sp.* in each concentration ($P< 0.05$). LSD analysis showed that growth of *Scenedesmus sp*. cultured in different concentration of tapioca wastewater (5%, 10%, 25%, 50%, 75%, and 100%) significantly differed from control treatment indicated by the green color (Figure 1). Unless of control treatment, logarithmic growth phase of *Scenedesmus sp.* started from 3 or 4 days, and exponential phase from 5 or 6 days (Figure 2). Compared with other previous studies on *Scenedesmus* growth [9,13,14] or even on the growth of *Chlorella phyrenoidosa* in tapioca wastewater [8], this study exhibits fast growth of *Scenedesmus* in every growth phase. It is probably due to the tapioca wastewater content (Table 1) that could support the growth of *Scenedesmus*.

Figure 1. (a). Growth profile of *Scenedesmus sp.* in different concentration of tapioca wastewater (b). Different concentration of *Scenesdemus sp*. culture in growth chamber

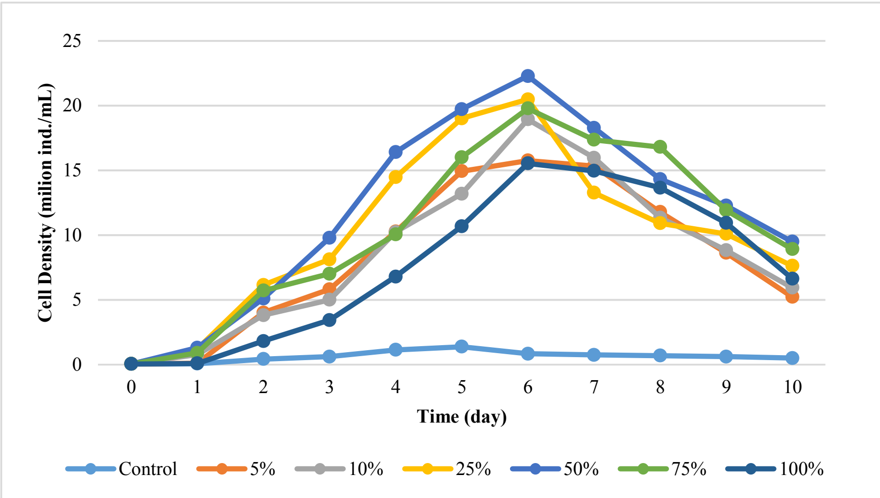

Figure 2. The exponential growth phase of *Scenedesmus sp*. shown by enhancement of *Scenedesmus sp*. cell density

We also determined the effect of tapioca wastewater treatment on lipid production at the end of growth culture of of *Scenedesmus sp.* (day 10). The different concentration of tapioca wastewater sample also affected the lipid production of *Scenedesmus sp.* ($P< 0.05$). LSD test revealed that *Scenedesmus sp.* which was cultured 50% of tapioca wastewater sample give highest lipid production than that of the other concentration (Figure 2). If we compare the ability of *Scenedesmus sp.* cultured in tapioca wastewater sample to produce lipid concentration, this study was highest than that of reported by Voltolina[14] which is only 12 – 14% of lipid content, or *Scenedesmus obliquus*[9] which is only 12 and 25%.

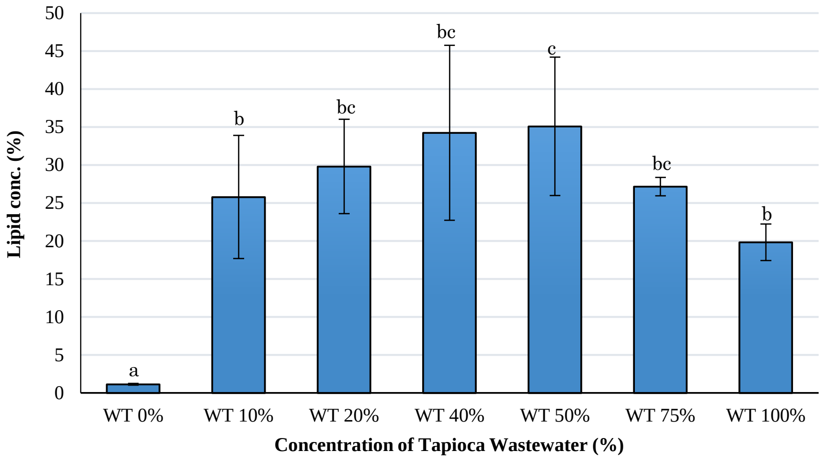

Figure 3. Lipid concentration of *Scenedesmus sp.* (%) cultured in different concentration of tapioca wastewater at day 10. Different letters indicate a significant different of lipid concentration produced by *Scnedesmus sp.* cultured in different concentration of tapioca wastewater at the level $p < 0.05$

The BOD, COD and nitrogen level of tapioca wastewater sample used in this study was higher than that of reported by previous researchers [3–5,14] that only around 10 – 40 mg/L of each parameter. However, the culture of *Scenedesmus sp.* in 100% of tapioca wastewaterdecreased BOD, COD and nitrogen level of wastewater, more than expected level of removal (Table 2). The addition of *Scenedesmus sp.*could decrease BOD and COD level of 74% and 72 %, respectively. This ability of *Scenedesmus sp.* in reducing of BOD and COD values in tapioca wastewater are higherthan previous study reported by Carvalho *et al* [5]. Furthermore, addition of *Scenedesmus sp.*culture in tapioca wastewater could also decrease pollutant level of NH_4, NO_3 and NO_2up to 92, 90 and 95% respectively, compared without addition/inoculation of *Scenedesmus sp.* These decreasing values of pollutant level reveal significant difference compared to previous study reported by other researches that *Scenedesmus sp.* could reduce only 40 to 70% of nitrogen residues in each wastewater samples [4,14].

To the best of our knowledge, this is the first report on the ability of *Scenedesmus* to produce high concentration of lipid and also decrease the pollutant level (more than standard values that stated by the regulation of Ministry of Environment, Republic of Indonesia), that might increase our understanding on the use of tapioca wastewater on microalgae production as well as to tackle environmental problem due to tapioca wastewater pollution.

Table 2. Nutrient removal level indicated by the decreasing of BOD, COD, NH_4, NO_3 and NO_2 level after addition of *Scenedesmus sp.* in tapioca wastewater.

Index	**Initial conc.**	**With *Scenedesmus sp.***		**Without*Scenedesmus* *sp.***		**Stand.***
		Value	**% rem.**	**Value**	**% rem.**	
BOD (mg/L)	245.09	62.98	74	239.54	2	200
COD (mg/L)	499.22	137.60	72	436.35	13	400
N-NH_4 (mg/L)	34.84	2.82	92	30.89	11	4
N-NO_3 (mg/L)	269.94	26.43	90	197.26	27	30
N-NO_2 (mg/L)	85.78	4.13	95	26.10	70	5
pH	4 - 5	7 – 8		5 - 6		6-9

* Standard value stated by regulation of Ministry of Environment, republic of Indonesia

4. Conclusions

It could be concluded that *Scenedesmus sp.* could growth in different concentration of tapioca wastewater sample, in which concentration 50% give best growth and highest concentration of total lipid than that of the other concentration. The level of environment indicator as nutrient removal such as BOD, COD, NH_4, NO_2, NO_3 were also decreased up to 74%, 72%, 95%, 91%, and 91%, respectively, as well as the pH condition.

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