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COMPARISON OF GROWTH MEDIUM BIOCONVERSION INTO *VANDA TRICOLOR* BIOMASS IN THIN LAYER LIQUID MEDIUM CULTURE SYSTEM AND TIS RITA® BIOREACTOR

Annisa Ratna Nurillah, Rizkita Rachmi Esyanti & Robert Manurung
Bioengineering Department, School of Life Sciences and Technology
Institut Teknologi Bandung (ITB)
annisaratna14@gmail.com
corresponding author: rizkita@sith.itb.ac.id

***Vanda tricolor* is one of the most valuable ornamental orchid species. However, there is a time constrain and some difficulties if Vanda is cultivated in conventional way. *In vitro* culture technique is an alternative solution to produce large quantity of Vanda shoot in relatively short period; i.e. by using system of TIS (temporary immersion system) RITA© and flask thin layer medium culture. This research was conducted to apply the TIS method to propagate Vanda shoot for the first time, as well as to evaluate the effect of differences in immersion period of the medium to the growth of culture and medium bioconversion into shoot biomass production.**

Shoot cultures of Vanda were grown in MS medium (half strength) with the addition of coconut water as a source of growth hormone, and performed using 2 RITA reactors with two different immersion period, 5 minutes and 10 minutes every 12 hours with incubation period of 21 days. Thin-layer culture system using same composition as in RITA system was performed in the flask with volume of 5 ml, agitated at a rate of 100 rpm, and incubated for 21 days.

The result of this research indicated that the orchid shoots could be cultured using TIS RITA bioreactor systems and thin-layer in flask system. Differences of culture immersion period affected growth rate. TIS RITA system with 5-minute immersion period produced greater growth rate (0.013 g/day) compared to that immersed for 10-minute (0.009 g/day), and thin-layer culture in flask showed the lowest growth rate, which was 0.012 g/day. The results of measurement of the sugar content and the conductivity of the medium showed the potential of medium conversion into biomass, and Vanda culture in TIS RITA system tent to grow more efficiently (growth rate of 0.013 g/day, 21% of sugar consumption) compared to that in flask system (growth rate of 0.012 g/day, 39% of sugar consumption).

Key words: *Vanda tricolor*, thin-layer culture system, TIS RITA, immersion period, growth rate, medium bioconversion.

I. INTRODUCTION

Orchids are one of decorative plants commodity with high economic value. Based on data from United States Department of Agriculture in 2006, orchid was included as a high value-potted plant with market value of $144 million in 2005. However, orchids especially *Vanda tricolor* tend to take a long period to grow and limit the possibility of its sustainable market supply.

Orchid cultivation could be done by various methods, including the conventional cultivation methods and in vitro cultivation. Conventional orchid cultivation begins with the germination process of seed and followed by incubation of seedling. Those processes take a long growth period and require a symbiosis with mycorhizal fungi [1]. Therefore an alternative method to cultivate the orchid effectively in efficient growth condition is needed, i.e. in vitro culture method. Tissue culture in thin layer liquid medium (in flask) and tissue culture in bioreactor are common in vitro culture method for biomass propagation, but they are distinguished in several aspects, including the capacity of system and the immersion period of culture by medium. Bioreactor system typically used for large quantities production of embryos in relatively short time, and bioreactor TIS (temporary immersion system) was an example of a commonly used bioreactor. The orchid culture in TIS bioreactor would not be submerged continuously [2], whereas in thin layer liquid medium system would continuously submerge. Therefore, this study was conducted to compare the potential of cultivating *Vanda tricolor* shoots in TIS RITA bioreactor and flask system, by evaluating the influence of various immersion periods on the shoot growth rate and the rate of nutrient uptake by *Vanda tricolor* biomass during cultivation.

II. MATERIALS AND METHODS

A. Medium preparation for Vanda tricolor culture

Medium was consisted of half-strength MS salts, 15 $ml.l^{-1}$ coconut water as growth hormone, and 30 $g.l^{-1}$ sucrose. The pH of medium was adjusted to 5.8 and autoclaved at 121 ^{0}C for 45 minutes. Then, the sterilized media was added by antibiotic (cefotaxime) as much as 100 ppm.

B. Thin layer liquid medium culture in flask.

Vanda tricolor shoots for thin layer liquid medium culture were obtained from solid medium culture. 10 shoots of Vanda were cultivated in 5 ml of medium and incubated at gyratory shaker and shaken at 100 rpm at room temperature (25^{0}C) for 21 days, under the light exposure 12-h per day.

C. Culture in TIS RITA bioreactor

Shoots were selected from thin layer liquid medium culture and cultivated in 250 ml media. Bioreactor was assembled and the airflow was controlled by timer with two variations, which gave two different immersion periods: (1) 5 minutes immersion time; (2) 10 minutes immersion time, every 12-h.

D. Plantlet and medium analysis

After 21 days cultivation, plantlets were harvested, weighed, and then dried to obtain the dry weight data. Sucrose remaining content and conductivity of culture medium were also tested. The conductivity was measured by using a conductivity meter (Eutech Instruments Con-110) and the test

of sucrose content in the medium was carried out by using a handheld refractometer (Atago U.S.A., Inc Uricon-NE).

E. Data analysis

Data of fresh weight were then transferred into growth curve. Data of sucrose content and conductivity of medium were used to construct a mass distribution model of Vanda shoot culture in flask system and in TIS RITA bioreactor.

The mass balance modeling was based on the following reaction:

$$aC_{12}H_{22}O_{11} + bNH_4NO_3 + cO_2 \rightarrow CH_{1.27}O_{0.43}N_{0.45} + dH_2O + eCO_2$$

Reaction coefficient for each compound was obtained from balancing the number of every element; such as carbon (C), hydrogen (H), oxygen (O), and nitrogen (N); and also used Gibbs energy balance [3]. The growth and sucrose consumption curves were developed based on the following equations [4]:

a. Growth kinetics

$$\frac{d}{dt}[X] = \mu \max X;$$

$$\mu = \mu max * ([C_{12}H_{22}O_{11}]/ (k_{C12H22O11} + C_{12}H_{22}O_{11}))$$

b. Sucrose consumption

$$d/dt\ [C_{12}H_{22}O_{11}] = - q_{C12H22O11} . X$$

$$q_{C12H22O11} = \mu / Y_{x/C12H22O11}$$

$$Y_{x/C12H22O11} = \Delta X / \Delta C_{12}H_{22}O_{11}$$

III. Results and Discussion

Observation result of *Vanda tricolor* shoots in flask indicated two different growth patterns in shoot culture, shoots elongation and shoots propagation [5]. The variation of growth pattern was influenced by the different culture period of shoots. Based on the data, growth in relation to shoots propagation (shoots multiplication) occurred in culture period of 45-65 days, while growth related to shoots elongation occurred in the period of 65-85 days.

Figures 1 and 2 showed the differences in the growth rate of culture in the flask and bioreactor. The highest growth rate value (0.013 g/day and 0.003 cm/day) was achieved by orchid shoots culture with 5 minutes of immersion period (RITA 1). While culture immersed for 10 minutes (RITA 2) had lower growth rate, which was 0.009 g/day and 0.006 cm/day, and shoots cultured in flask had growth rate of 0.012 g/day and 0.005 cm/day.

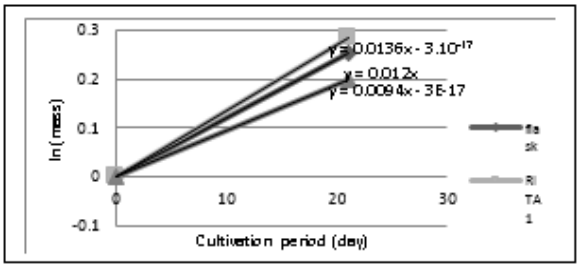


Figure 1. Graph of *Vanda tricolor* biomass production of shoots culture in bioreactor and flask system after 21 days of cultivation.

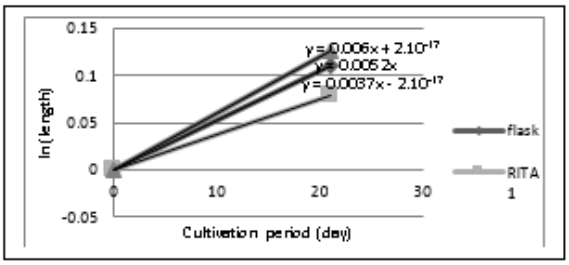


Figure 2. Graph of *Vanda tricolor* shoots elongation in bioreactor and flask system after 21 days of cultivation.

The Table 1 below showed that sugar consumption value of shoots culture in flask system was 39.3% with growth rate of 0.012 g/day, whereas sucrose consumption value of RITA 1 was smaller (21.1%), but had a higher growth rate (0.013 g/day), and RITA 2 had the lowest consumption value (6%) with the lowest growth rate of 0.009 g/day. Meanwhile, the conductivity reduction data showed a tendency that the more often explants in contact with the medium, the greater reduction of conductivity would be. However, the greater the conductivity reduction did not result in greater growth rates. This indicated that the ion transfer occurred, but did not effectively facilitate the transport of nutrients those needed by explants from the medium.

TABLE I. Growth kinetics and medium analysis data

Culture System	Growth Rate (g/day)	Shoots Elongation Rate (cm/ day)	Conductivity Reduction (mS)	Total Sugar Consumption (g)
RITA 1	0.013	0.003	0.66	7.8
RITA 2	0.009	0.006	1.44	2.2
Flask	0.012	0.005	6.775	14.4

The ion transfer probably rather to facilitate the transport of water from the medium, giving some symptoms of hyperhydricity or vitrification [6], such as shoots were glassy, the bottom part of the shoots blackened, and shoots had pale green color as seen in Figure 3.

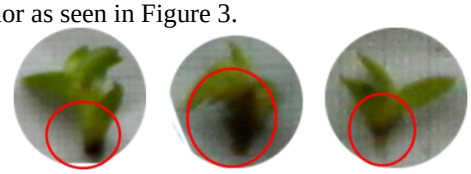


Figure 3. Shoots with hyperhydric symptoms.

Based on these data, the medium bioconversion phenomena in the system with continuous submersion culture did not occur efficiently, but system with short immersion period produced better efficiency for the bioconversion.

Based on the data in Tables 2, 3, and 4, the substrate in the form of carbon source ($C_{12}H_{22}O_{11}$) and nitrogen source (NH_4NO_3) was converted into biomass. The substrates bioconversion followed the reaction of respiration, in which the reaction coefficients were obtained from the element balance equation and Gibbs energy balance equation. The table showed the hypothetical amount of biomass and the entire value was different from the actual amount of biomass obtained from the measurement of plantlets weight. Amount of biomass in shoots culture in flask system was hypothetically 0.44 g and the amount of actual biomass was 0.17 g, with 14.44 g sucrose was consumed. The hypothetical amount of biomass in RITA 1 was 0.23 g while the actual was 0.32 g,

and the hypothetical amount of biomass in RITA 2 was 0.07 g while the actual was 0.28 g. Based on those data, the substrate consumption (sucrose) in TIS RITA bioreactor system was more efficient than in flask system, which for flask system, the hypothetical amount was lower than the actual biomass, while in the RITA system the actual biomass obtained was greater than the hypothetical amount.

TABLE II. RESPIRATION REACTION DATA FOR CULTURE IN FLASK SYSTEM

Reaction Equation	$0.39C_{12}H_{22}O_{11}$	$0.23NH_4NO_3$	$3.43O_2$	$CH_{1.27}O_{0.43}N_{0.45}$	$4.07H_2O$	$3.64CO_2$
reaction (g)	14.44	5.73	0.15	0.44	0.073	0.199
reaction (mol)	0.0422	0.072	0.0048	0.0165	0.004	0.0045

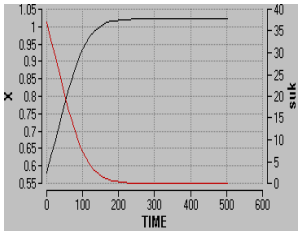
TABLE III. RESPIRATION REACTION DATA FOR CULTURE IN TIS RITA 1

Reaction Equation	$0.39C_{12}H_{22}O_{11}$	$0.23NH_4NO_3$	$3.43O_2$	$CH_{1.27}O_{0.43}N_{0.45}$	$4.07H_2O$	$3.64CO_2$
reaction (g)	7.7778	3.0850	0.0827	0.2346	0.0392	0.1072
reaction (mol)	0.0227	0.0386	0.0026	0.0089	0.0022	0.0024

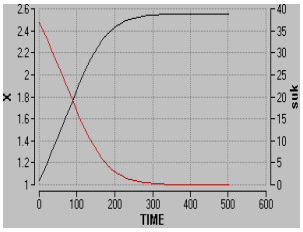
TABLE IV. RESPIRATION REACTION DATA FOR CULTURE IN TIS RITA 2

Reaction Equation	$0.39C_{12}H_{22}O_{11}$	$0.23NH_4NO_3$	$3.43O_2$	$CH_{1.27}O_{0.43}N_{0.45}$	$4.07H_2O$	$3.64CO_2$
reaction (g)	2.22	0.88	0.0236	0.067	0.0112	0.0306
reaction (mol)	0.0065	0.011	0.0007	0.0025	0.0006	0.0007

Figure 4 showed that every culture system had a curve intersection of sucrose consumption and biomass growth curve at different time. The curve was obtained from equation modeling of the growth kinetics and the consumption rate of sucrose with the help of Berkeley Madonna software. The results of the modeling showed that the flask culture system had a curve intersection at an earlier time than the RITA 1 and RITA 2 system. However, the maximum biomass amount of flask system (1 unit of biomass) was lower than the RITA 1 system (2.5 unit biomass) and RITA 2 (5.5 unit of biomass). Based on the analysis of the curve intersection and the maximum amount of biomass, the data showed that the orchid culture in TIS RITA system with 5 minutes immersion resulted in better ability of substrate (including sucrose) bioconversion into biomass than the same system with longer immersion period (10 minutes), and also more efficient than the flask system culture.



(a)



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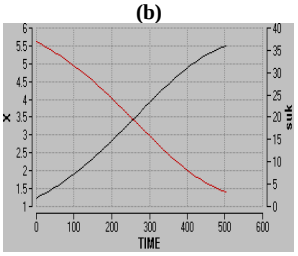

(c)

Figure 4. Sucrose consumption and biomass growth curves in (a) flask, (b) RITA 1, and (c) RITA 2 system.

IV. CONCLUSION

Shoots of *Vanda tricolor* cultured in TIS RITA bioreactors was potential to be developed as a better method of shoot production, compared with other methods such as thin layer liquid medium culture. The period of culture immersion was a parameter that directly affected the growth of orchids, where different immersion time would result in different growth rates. Five minutes immersion in medium was an optimal immersion period for growth of the *Vanda tricolor* shoots compared with that of 10 minutes in RITA bioreactor. Growth rate of culture in RITA bioreactor system with optimal immersion period (5 minutes) was higher compared to the thin-layer liquid medium culture system.

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