KINETIC MODEL OF COMMERCIAL GLUCOSE-AFFECTED GROWTH AND MICROBIAL OIL PRODUCTION OF OLEAGINOUS YEAST *Pseudozyma parantarctica* CHC28

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ABSTRACT

This research proposed to study the effect of commercial glucose concentration in a nitrogen-limiting medium on the growth of *Pseudozyma parantarctica* CHC28 and to estimate the kinetic parameters of fermentation. The biomass, microbial oil, biomass production rate (Q_x), and oil production rate (Q_p) increased dramatically and reached 22.10±1.95 g/L, 10.99±1.31 g/L, 0.184±0.016 g/L h, and 0.092±0.011 g/L h, respectively, when glucose concentration was increased to 100 g/L (C/N ratio = 333). In contrast, these results showed a decrease when glucose and C/N ratio were higher than 100 g/L and 333, respectively. Concurrently, the oil content showed a continuous rise as the glucose and C/N ratio increased. Furthermore, the maximum specific growth rate (μ_{max}) declined sharply when *P. parantarctica* was cultivated under high glucose concentration. It was therefore suggested that the glucose concentration could affect the growth of oleaginous yeast because of osmotic pressure and C/N ratio. For the bioreactor scale, biomass, microbial oil, and oil content were enhanced to 29.92 g/L, 15.13 g/L, and 50.57 %w/w, respectively, at 120 hrs of cultivation. The mathematical models could describe the effect of glucose concentration on both yeast growth and microbial oil production. Thus, the kinetic model satisfactorily fitted the experimental data relating to oleaginous growth, microbial oil production, and substrate consumption.

Keywords: oleaginous yeast, microbial oil, glucose-affected growth, kinetic model

INTRODUCTION

The energy crisis is one of the most critical issues in current times. Due to the limited reserves of fossil fuels, high energy demand, and environmental impact, meeting the future energy demand is a matter of great concern (**Ma *et al*., 2018**). Renewable energy has emerged as one of the most feasible solutions to overcome this challenge. Biodiesel obtained from energy crops and their derivatives reduces undesirable effects on the environment, such as acid rain and the greenhouse effect caused by fossil fuel combustion. In addition, it offers an advantage over fossil fuels because it is biodegradable and renewable (**Soccol *et al*., 2017**). However, the amount of vegetable oils available for biodiesel production is insufficient in case of increase in demand. Currently, single-cell oil or microbial oil has been reported to be a fascinating alternative source for biodiesel production. The cultivation of microorganisms producing microbial oil can be carried out through an uncomplicated process with low-cost raw materials. The microorganism which accumulates more than 20 percent of intracellular oil is identified as an oleaginous microorganism (**Qin *et al*., 2017**). The various oleaginous microorganisms which can produce microbial oil include bacteria, yeasts, molds, and microalgae (**Qasim and Sultan, 2020; Saenge *et al*., 2011**). Previous research has shown that yeast has an advantage over other oleaginous microorganisms, in growing and producing oil at a higher rate (**Saenge *et al*., 2011**). The fatty acid compositions of yeasts are similar to that of vegetable oils such as palm, coconut, jatropha, and rapeseed oils (**Ong *et al*., 2011; Satyanarayana and Muraleedharan, 2011; Areesirisuk *et al*., 2015; Sutanto *et al*., 2018**). Oleaginous yeast is cultured in order to extract intracellular oil from microbial cells. The microbial oils thus obtained are used as a feedstock for biodiesel production, thus offering an interesting alternative (**Li *et al*., 2007**). Previous research has found that oleaginous microorganisms could be induced to produce and accumulate oil in media containing an excess of carbon, with

limitation in the quantities of some elements such as phosphorus, sulfate, or nitrogen (**Gill *et al*., 1977; Jakobsen *et al*., 2008; Wu *et al*., 2010, 2011; Bonturi *et al*., 2017**). For instance, phosphorus is an important component of microbial growth. It is used to determine the composition of biomolecules, i.e., nucleic acid, phospholipids, and coenzymes. Studies show that phosphate limitation could lead to oil production in oleaginous yeast (**Granger *et al*., 1993; Wu *et al*., 2010**). Further, nitrogen deficiency was also proposed as a master regulator of oil synthesis (**Ratledge and Wynn, 2002; Bonturi *et al*., 2017**). Moreover, it has been observed that the application of nitrogen starvation strategy in microbial oil production, provides higher intracellular oil content when compared to oil content generated by the limitation of other elements (**Wu *et al*., 2010**).

In this context, a mathematical model of the microbial fermentation process may be applied to understand and predict the fermentation profile. Moreover, such models could be used to estimate the kinetic parameters of fermentation. For instance, a practical model could be used to at least indicate parameters such as microbial growth, substrate consumption, and characteristics of product formation during fermentation (**Yang *et al*., 2011; Farias *et al*., 2014**).

Therefore, given all of the above-mentioned factors, in this study, kinetic modeling was used to evaluate the effect of glucose-based nitrogen-limiting media on growth, microbial oil production, and substrate consumption of oleaginous yeast *Pseudozyma parantarctica* CHC28. This research aimed to study the influence of commercial glucose concentration on the growth of oleaginous yeast and to employ a mathematical model for predicting yeast growth and microbial oil production profiles.

MATERIALS AND METHODS

Yeast strain and inoculum preparation

P. parantarctica CHC28, an oleaginous yeast, was used in this study (**Areesirisuk *et al.*, 2015**). The stock culture of oleaginous yeast CHC28 was re-cultured in Yeast Malt broth (YM broth) at 30°C, 150 rpm of shaking speed, for 24 hrs. Subsequently, 5 mL volume of activated yeast (with an optical density of approximately 0.7-0.8 at 600 nm) culture was transferred to a 100 mL seed medium containing (in g/L): glucose 20, yeast extract 0.5, $(NH_4)_2SO_4$ 5.0, $MgSO_4 \cdot 7H_2O$ 0.5, and KH_2PO_4 1.0. It was cultured as per the above-mentioned procedure for 20 hrs. This cultured yeast was used as a starter in the following experiment.

Glucose-affected growth of oleaginous yeast

Five milliliters of yeast starter were aseptically inoculated into 100 mL of nitrogen-limiting medium containing (in g/L): yeast extract 0.1, $(NH_4)_2SO_4$ 0.5, $MgSO_4 \cdot 7H_2O$ 0.15, and KH_2PO_4 7.0. Commercial glucose produced from cassava starch (cassava starch-based glucose, WGC, Co. Ltd.) was used as the primary carbon source. This glucose was added as the sole carbon source and its concentration was varied between 20 to 300 g/L. The batch fermentation was performed at 30°C and 150 rpm of shaking speed. The culture was withdrawn aseptically from the experimental flask and the biomass, oil, and glucose concentration were determined as described below. The experiments were run in triplicates.

The bioreactor scale experiment was performed using 5 L of nitrogen-limiting medium containing glucose 100 g/L in a 10-L fermenter (Major Science, MS-F1, Taiwan). Fifteen milliliters of the re-cultured oleaginous strain were aseptically transferred to 300 mL of seed medium and cultured at 30°C and 150 rpm of shaking speed for 20 hrs. Five hundred milliliters of seed culture were aseptically inoculated to the bioreactor and cultivated at 30°C, a stirring speed of 300 rpm, and 1.0 vvm of aeration rate. The sample was withdrawn throughout cultivation to determine glucose, biomass, and oil concentration.

Analytical method

The yeast cells were harvested by centrifugation at 12,000 rpm for 10 min and washed twice with water. The cleaned cells were dried at 60°C to a constant weight, usually for 24 hrs. The biomass was determined gravimetrically and expressed as the gram of biomass per liter (**Xue *et al.*, 2008**). The oil concentration was analyzed with a mixture of chloroform and methanol, according to the method proposed in **Bligh and Dyer (1959)**. Glucose was determined using 3,5-dinitrosalicylic colorimetric method (**Miller, 1959**).

Kinetic model

The growth rate of oleaginous yeast was investigated by the Logistic equation in (1) (**Zajšek and Goršek, 2010; Laopaiboon *et al.*, 2016**), as follows:

$$\frac{dX}{dt} = \mu_{max} X \left(1 - \frac{X}{X_{max}}\right) \qquad (1)$$

where μ_{max} was the maximum specific growth rate, X was the biomass at specific times (t) of yeast growth, and X_{max} was maximum biomass concentration.

The rate of substrate consumed (dS/dt) was used to explain the cell growth rate (including the intracellular oil) and the biomass concentration following the equation (2):

$$\frac{dS}{dt} = \frac{1}{Y_{x/s}} \left(\frac{dX}{dt}\right) + m_s \qquad (2)$$

where $Y_{x/s}$ was the biomass yield on substrate and m_s was the biomass maintenance coefficient. The rate of production of oil (dP/dt) was dependent on the biomass concentration, following the Luedeking-Piret equation given below:

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \qquad (3)$$

In Eq. (3) α was a growth-associated oil formation coefficient, and β was a non-growth-associated oil formation coefficient. If $\alpha \neq 0$ and $\beta = 0$, the oil formation was directly coordinated to microbial growth; if $\alpha \neq 0$ and $\beta \neq 0$, the oil formation

was partially dependent on microbial growth; and if $\alpha = 0$ and $\beta \neq 0$, the oil formation was unrelated to microbial growth (**Gaden, 2000; Yang *et al.*, 2011**).

Estimation of model parameters

The kinetic parameters of the Logistic model (μ_{max} and X_{max}) were calculated to fit the model and the experimental data. The fitting was performed using Berkley Madonna™ software for curve fitting (**Marudkla *et al.*, 2018**). The coefficient of determination (R^2) was applied to measure the model fitting quality between the predicted and the experimental data (**Wannawilai and Sirisansaneeyakul, 2015**). R^2 was calculated using the following equation:

$$R^2 = \frac{\Sigma(C_{pred} - \bar{C}_{exp})^2}{\Sigma(C_{pred} - \bar{C}_{exp})^2 + \Sigma(C_{pred} - C_{exp})^2} \qquad (4)$$

In Eq. (4), C_{pred} was the value of a predicted variable using the mathematical model. C_{exp} and $\bar{C}_{exp}$ were the corresponding experimental values and the average of all the experimented values of the critical variable, respectively. The non-normalized root means square ($NRMS$) value was employed to investigate the actual error for each determination (**Wannawilai and Sirisansaneeyakul, 2015**). The $NRMS$ was expressed as follows:

$$NRMS = \sqrt{\frac{\Sigma_{i=1}^{N}(C_{cal} - C_{exp})^2}{N}} \qquad (5)$$

where N is the number of determinations.

Statistical method

The experimental data were examined for statistical significance using a one-way analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was used to compare the effect of glucose on fermentation parameters. Statistical analysis was performed using SPSS 15.0 software (SPSS Inc., USA).

RESULTS AND DISCUSSION

Glucose-affected growth and oil production of oleaginous yeast

The glucose-affected growth of *P. parantarctica* CHC28 was studied. The batch fermentations were operated in a shaking flask with glucose variation between 20 g/L and 300 g/L. Figure 1 represents the biomass concentration of *P. parantarctica* CHC28 against cultivation time. The results showed that the growth of *P. parantarctica* CHC28 was affected by glucose concentration as the primary carbon source. The yeast growth increased with an increase in glucose and then decreased slightly when glucose was increased to 120 g/L. This result suggested that *P. parantarctica* CHC28 could grow well without substrate inhibition under a wide range of substrate concentrations, especially from 20 to 100 g/L.

Table 1 presents the microbial oil production of yeast following 120 hrs of cultivation. The biomass and oil production were enhanced following the increase in the glucose concentration until 100 g/L. Subsequently, they clearly dropped when yeast was cultured in glucose over 100 g/L. The oil content increased continuously in accordance with the increasing C/N ratio, and the maximum oil content of 59.70±2.51% was obtained at 300 g/L of glucose (C/N ratio = 999). Simultaneously, the biomass production rate (Q_x) and oil production rate (Q_p) rapidly reached the highest values of 0.190±0.019 and 0.092±0.011 g/L at glucose levels of 80 and 100 g/L, respectively. Furthermore, the Q_x and Q_p reduced dramatically when glucose was higher than 100 g/L. Thus, it was found that when *P. parantractica* CHC28 was cultured in glucose 100 g/L (C/N ratio = 333), it produced the maximum biomass, oil, Q_x, and Q_p, at the rate of 22.10±1.95 g/L, 10.99±1.31 g/L, 0.184±0.016 g/L h, and 0.092±0.011 g/L h, respectively. These results suggested that oil accumulation occurred most frequently, when the nutrient source, particularly the nitrogen source, was limited, while the excess carbon source was offered at the required levels (**Bonturi *et al.*, 2017**). Typically, it occurred concurrently with the stationary phase. Thus, nitrogen limitation has proved to be one of the most practical methods to induce oil production because it can easily control media composition. Eventually, oil synthesis pathways of microorganisms were positively regulated to balance cell growth with energy storage (**Beopoulos *et al.*, 2009; Tai and Stephanopoulos, 2013**). Also, earlier studies have also supported the well-known fact that optimum C/N ratio can repress Kreb's cycle performance and accumulate citric acid for microbial oil synthesis (**Huang *et al.*, 2016**).
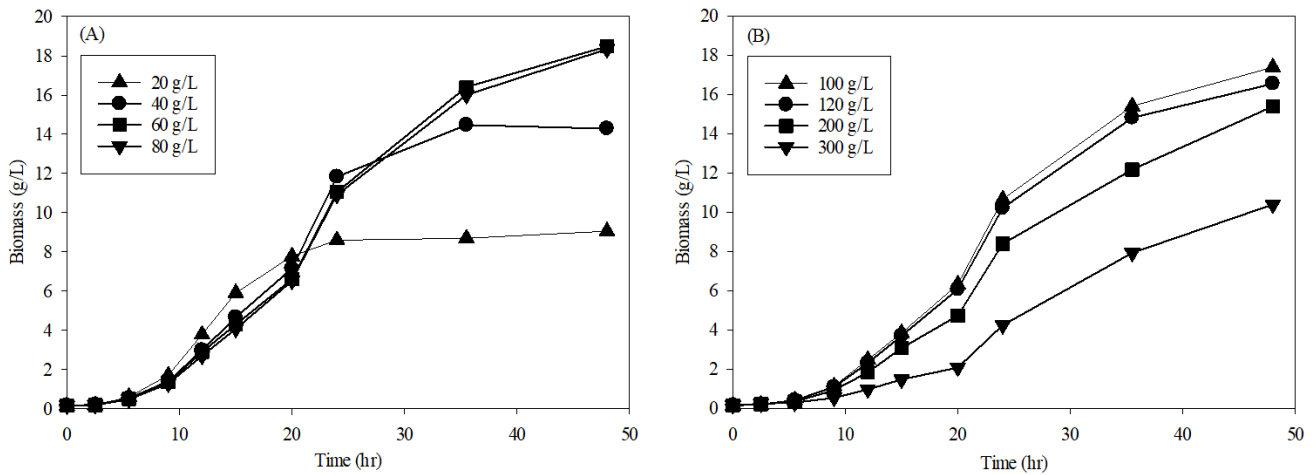
Figure 1 Biomass growth during fermentation in the glucose-based nitrogen-limiting medium. The initial glucose concentrations were (A) 20-80 g/L and (B) 100-300 g/L.

Table 1 Effect of initial glucose concentration on biomass, oil, oil content, biomass production rate, and oil production rate of *P. parantarctica* at 120 hrs of cultivation

Glucose (g/L)	C/N ratio	Biomass (g/L)	Oil (g/L)	Oil content (%w/w)	Q_x (g/L h)	Q_p (g/L h)
20	67	9.11±0.33d	1.09±0.31e	11.83±3.04f	0.076±0.003d	0.009±0.003e
40	133	15.24±0.10c	3.43±0.43d	22.52±2.81e	0.127±0.001c	0.029±0.004d
60	200	21.02±0.12ab	6.96±0.49c	33.10±2.17d	0.175±0.001ab	0.058±0.004c
80	267	22.85±2.28a	9.34±1.17ab	41.48±9.24c	0.190±0.019a	0.078±0.010b
100	333	22.10±1.95ab	10.99±1.31a	49.64±1.54b	0.184±0.016ab	0.092±0.011a
120	400	20.27±1.68b	9.78±1.74ab	48.03±4.83bc	0.169±0.014b	0.082±0.015ab
200	666	17.15±0.12c	8.78±0.08b	51.21±0.66b	0.143±0.001c	0.073±0.001b
300	999	9.80±0.51d	5.84±0.08c	59.70±2.51a	0.082±0.004d	0.049±0.001c

Legend: The data indicate mean ± standard deviation (SD). The different letters alongside the values in the same column indicate significant differences (*p*-value < 0.05).

Modeling the growth kinetic

A growth kinetic model was used to predict the behavior of the microbial cultivation process. Equation (1) was deployed to model the biomass growth in nitrogen-limiting media, which contained glucose as the major carbon source. During cultivation, the lag phase was observed during 6-11 hours of fermentation. Subsequently, the *P. paranrarctica* CHC28 presented a classical growth trend corresponding to the sigmoid curve. Moreover, it was found that the final biomass concentration also depended on the initial glucose concentration. At the end of fermentation, the microbial oil was produced at an increasing rate when the initial substrate was enhanced to 100 g/L and decreased slightly when glucose levels were raised higher than 120 g/L.

As shown in figures 2A-2H, the Logistic model fitted the experimental data well (determination coefficients (R^2) ≥ 0.9907 and *NRMS* ≤ 0.4920). The biomass of *P. parantarctica* CHC28 could be satisfactorily modeled. As can be seen, the R^2 values obtained from the Logistic model were very high. All R^2 values were

higher than 0.99. This result demonstrated that the Logistic model was suitable for describing the experimental biomass profile. However, it should be noted carefully that the Logistic equation could not explain the lag time and maximum biomass productivity.

Further, the Logistic model is ideal for estimating the growth kinetics, i.e., the maximum specific growth rate (μ_{max}, h⁻¹) **(Phukoetphim *et al.*, 2017)**. Table 2 shows the kinetic values in the Logistic model. The μ_{max} was used to define the glucose-affected growth of *P. parantarctica* CHC28. It revealed that the highest μ_{max} was obtained by using glucose 20g/L for 0.333±0.022 h⁻¹. Subsequently, the μ_{max} decreased dramatically upon cultivation in the nitrogen-limiting medium containing high initial glucose concentration, though the μ_{max} of yeast cultivated in glucose 60-120 g/L was not significantly different (*p*-value < 0.05). The cultivation of *P. parantarctica* in glucose 300 g/L provided the lowest μ_{max} for 0.146±0.004 h⁻¹. These results suggested that glucose concentration and C/N ratio could affect oleaginous yeast growth because of osmotic pressure.

Table 2 The kinetic values in the Logistic model of glucose-affected growth of oleaginous yeast *P. parantarctica* CHC28

Glucose (g/L)	μ_{max} (h⁻¹)	X_{max} (g/L)	R^2	*NRMS*
20	0.333±0.022a	8.81±0.19e	0.9985	0.1360
40	0.223±0.005b	15.31±1.18c	0.9929	0.4638
60	0.178±0.003c	18.41±0.20a	0.9962	0.3960
80	0.180±0.002c	18.15±0.16a	0.9961	0.3972
100	0.189±0.004c	17.19±0.25b	0.9964	0.3647
120	0.192±0.006c	16.39±0.44b	0.9968	0.3299
200	0.161±0.002d	15.17±0.01c	0.9907	0.4920
300	0.146±0.004e	10.93±0.56d	0.9963	0.2061

Legend: The data indicate mean ± standard deviation (SD). The different letters alongside the values in the same column indicate significant differences (*p*-value < 0.05).
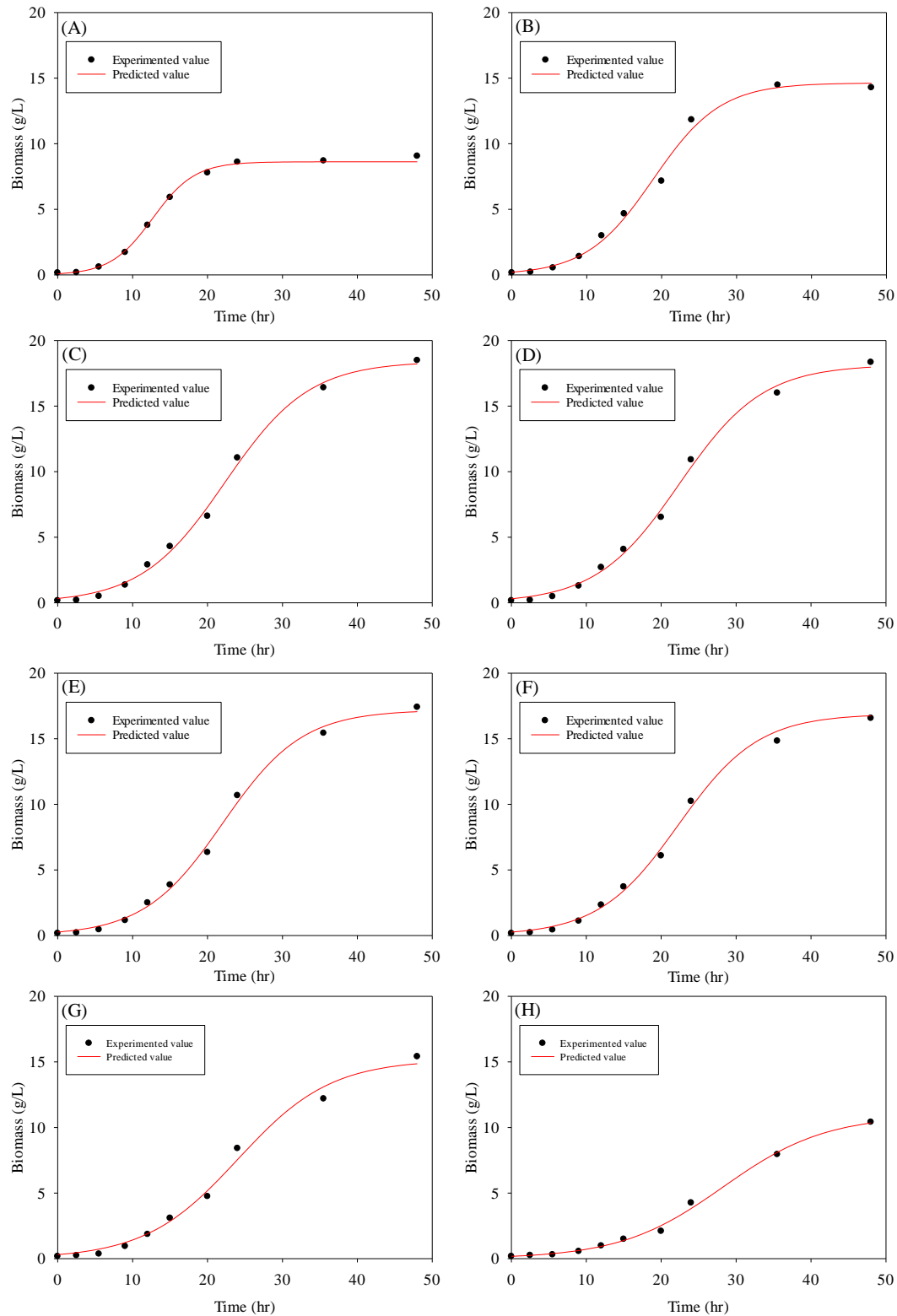
Figure 2 The experimental values (symbols) and model predictions (lines) of biomass concentration in the flask scale. Glucose-based medium with following initial concentrations of : (A) 20 g/L; (B) 40 g/L; (C) 60 g/L; (D) 80 g/L; (E) 100 g/L; (F) 120 g/L; (G) 200 g/L; (H) 300 g/L.

Scale-up of microbial oil production in bioreactor and its kinetic modeling

The fermentation profile of oleaginous yeast in a bioreactor (glucose 100 g/L, C/N ratio = 0.333) was illustrated in figure 3. The lag time was verified using the online DMFit software (http://browser.combase.cc/DMFit.aspx) to fit a curve to the experimental biomass value. The result presented a lag phase of 8.14 hrs, which was shorter than that in a flask scale experiment (9.65 hrs for glucose 100 g/L), following which it entered the log and stationary phase. The maximum biomass was provided at 18.96 g/L for 48 hrs, which was higher than that in the flask scale (17.19 g/L). Besides, at 120 hrs of cultivation, the biomass and oil

levels reached 29.92 g/L and 15.13 g/L, respectively. At the same time, the intracellular oil accumulation reached 50.57 %w/w. A comparison of the growth rate and oil production efficiency demonstrated that the biomass, oil, and oil content in the bioreactor scale were higher than those in the flask scale. The cause for this difference may be aeration. Previous studies have reported the influence of different aeration supplies in flask and bioreactor scale experiments on oleaginous yeast growth and microbial oil production (**Yen and Zhang, 2011; Yen and Liu, 2014; Karamerou *et al.*, 2016**). It was demonstrated that the growth and oil yield of *Rhodotorula glutinis* showed an apparent increase in baffled bioreactor compared with a non-baffled bioreactor, indicating that air

supply plays a key role in the growth of *R. glutinis*. However, when the aeration rate was increased higher than 0.5 L/min, the cell and oil concentrations were reduced. According to these findings, higher aeration did not improve growth or oil production. Although the impacts of aeration rates are still unclear, the size of air bubbles in the bioreactor might be a possible influencing factor. Higher airflow rates produce larger air bubbles with a smaller specific surface area, resulting in less oxygen mass transfer (**Karamerou *et al*., 2016**). However, the effect of oxygen supply on the growth and oil production of *P. parantarctica* CHC28 should be further investigated.
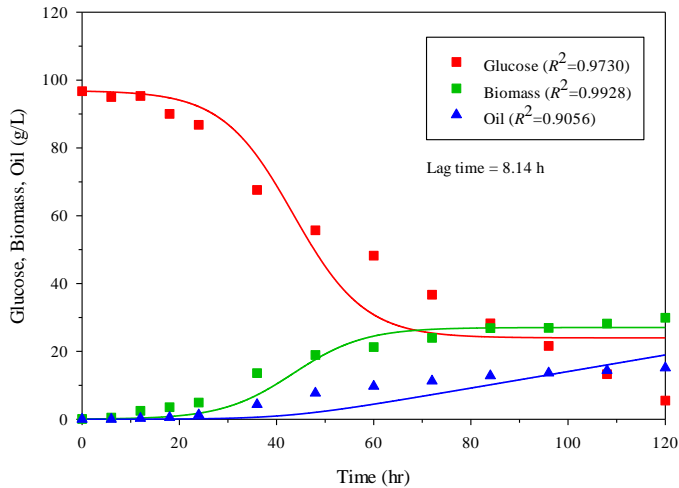

Figure 3 The experimental values (symbols) and model predictions (lines) of glucose, biomass, and oil concentration during fermentation with 5 L nitrogen-limiting medium containing glucose 100 g/L in a bioreactor.

The values of the best-fit model parameters (i.e., μ_{max}, X_{max}, $Y_{x/s}$, m_s, α, and β) in the bioreactor were shown in Table 3. The μ_{max} was 0.137 h^{-1}, which is lower than the flask scale. However, the X_{max} was higher than the shaking flask scale (27.03 g/L). The m_s value was nearly zero, indicating that the almost substrate was used to form the oleaginous biomass, which was higher than that used for cell maintenance. The α and β were very similar, which demonstrated that the oil

production of *P. parantarctica* CHC28 was partially dependent on microbial growth (**Yang *et al*., 2011**).

Table 3 The model-estimated fermentation parameters in a bioreactor

Model equations	Parameters	Values
$\frac{dX}{dt} = \mu_{max} X \left(1 - \frac{X}{X_{max}}\right)$	μ_{max} (h^{-1})	0.137
	X_{max} (g/L)	27.03
	R^2	0.9928
	$NRMS$	0.3287
$\frac{dS}{dt} = -\left[\left(\frac{1}{Y_{x/s}}\right)\left(\frac{dX}{dt}\right) + m_s X\right]$	$Y_{x/s}$	0.37
	m_s (g/g h)	3.12 x 10^{-10}
	R^2	0.9730
	$NRMS$	2.9693
$\frac{dP}{dt} = (\alpha\mu + \beta) X$	α	0.0091
	β	0.0090
	R^2	0.9056
	$NRMS$	0.4484

Table 4 presents a comparison of different carbon sources and C/N ratios for microbial oil production. In this study, the biomass and oil concentrations were obtained for 22.10 and 10.99 g/L respectively, in the flask scale experiment. The oil concentration was lower than gained by **Zhu *et al*. (2008)** using *Trichosporon fermentans* (13.80 g/L), cultured in glucose 100 g/L (C/N ratio = 140). The microbial oil production in bioreactor scale could enhance the biomass and oil production to a level higher than the flask scale and the results by **Zhu *et al*. (2008)** and **Bonturi *et al*. (2017)**. **Fakas *et al*. (2009)** studied the microbial oil production from *Cunninghamella echinulate* ATHUM 4411 and *Mortierella isabellina* ATHUM 2935, using xylose as the main carbon source with a similar C/N ratio (285). The results exhibited variation in microbial oil production efficiency. **Karamerou *et al*. (2016)** cultured the *Rhodotorula glutinis* in glycerol and achieved biomass and oil of 4.06 and 1.21 g/L, respectively, which led to lower oil content (29.80%). These results showed that microbial oil production depended on the carbon source with proper C/N ratio and the oleaginous strain.

Table 4 Studies of microbial oil production using different carbon sources and C/N ratios

Strains	Conditions	C/N ratio	Biomass (g/L)	Oil (g/L)	Oil content (%w/w)	Q_x (g/L h)	Q_p (g/L h)	References
Trichosporon fermentans CICC 1368	Glucose, 100 g/L	140	21.80	13.80	63.10	0.130[a]	0.082[a]	(**Zhu *et al*., 2008**)
Cunninghamella echinulate ATHUM 4411	Xylose, 80 g/L	285	7.80	4.50	57.70	0.041[b]	0.023[b]	(**Fakas *et al*., 2009**)
Mortierella isabellina ATHUM 2935	Xylose, 80 g/L	285	8.70	5.70	65.50	0.040[c]	0.026[c]	(**Fakas *et al*., 2009**)
Rhodotorula glutinis CICC 31596	Pure glycerol, 60 g/L	137	4.06	1.21	29.80	-	-	(**Karamerou *et al*., 2016**)
Rhodosporidium toruloides CCT 0783	Glucose, 70 g/L	95	16.50	7.90	47.90	-	0.083	(**Bonturi *et al*., 2017**)
P. parantarctica CHC28	Glucose (flask), 100 g/L	333	22.10	10.99	49.64	0.184	0.092	This study
	Glucose (bioreactor), 100 g/L	333	29.92	15.13	50.57	0.250	0.130	This study

Legend: [a,b,c]The data were calculated with cultivation times of 168, 192, and 219 hrs, respectively; - : Information not available.

CONCLUSION

A glucose concentration composed in a nitrogen-limiting medium affected the growth and microbial oil production of *P. parantarctica* CHC28. The increase in glucose concentration along with the C/N ratio enhanced biomass and oil concentration. However, the maximum specific growth rate was decreased in a high glucose concentration due to the osmotic pressure in the solution. The oil content of 50.57 %w/w was obtained in the bioreactor. The mathematical model employed in this study could efficiently describe the experimental data in both flask and bioreactor scales. The R^2 and $NRMS$ values were higher and lower than 0.9056 and 2.9693, respectively.

REFERENCES

Areesirisuk, A., Chiu, C. H., Yen, S. B., & Guo, J. H. (2015). A novel oleaginous yeast strain with high lipid productivity and its application to alternative biodiesel production. *Applied Biochemistry and Microbiology*, *51*(4), 411–418. https://doi.org/10.1134/s0003683815030035

Beopoulos, A., Cescut, J., Haddouche, R., Uribelarrea, J.-L., Molina-Jouve, C., & Nicaud, J.-M. (2009). *Yarrowia lipolytica* as a model for bio-oil production. *Progress in Lipid Research*, *48*(6), 375–387. https://doi.org/10.1016/j.plipres.2009.08.005

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911–917. https://doi.org/10.1139/o59-099

Bonturi, N., Crucello, A., Viana, A. J. C., & Miranda, E. A. (2017). Microbial oil production in sugarcane bagasse hemicellulosic hydrolysate without nutrient supplementation by a *Rhodosporidium toruloides* adapted strain. *Process Biochemistry*, *57*, 16–25. https://doi.org/10.1016/j.procbio.2017.03.007

Fakas, S., Papanikolaou, S., Batsos, A., Galiotou-Panayotou, M., Mallouchos, A., & Aggelis, G. (2009). Evaluating renewable carbon sources as substrates for single cell oil production by *Cunninghamella echinulata* and *Mortierella isabellina*. *Biomass and Bioenergy*, *33*(4), 573–580. https://doi.org/10.1016/j.biombioe.2008.09.006

Farias, D., Andrade, R. R. de, & Maugeri-Filho, F. (2014). Kinetic modeling of ethanol production by *Scheffersomyces stipitis* from xylose. *Applied Biochemistry and Biotechnology*, *172*(1), 361–379. https://doi.org/10.1007/s12010-013-0546-y

Gaden, E. L. (2000). Fermentation process kinetics. Reprinted from Journal of Biochemical Microbiological Technology and Engineering Vol. 1, No. 4 Pages 413-29 (1959). *Biotechnology and Bioengineering*, *67*(6), 629–635.

Gill, C. O., Hall, M. J., & Ratledge, C. (1977). Lipid accumulation in an oleaginous yeast (Candida 107) growing on glucose in single-stage continuous culture. *Applied and Environmental Microbiology*, *33*(2), 231–239. https://doi.org/10.1128/aem.33.2.231-239.1977

Granger, L.-M., Perlot, P., Goma, G., & Pareilleux, A. (1993). Effect of various nutrient limitations on fatty acid production by *Rhodotorula glutinis*. *Applied Microbiology and Biotechnology*, *38*(6), 784–789. https://doi.org/10.1007/BF00167145

Huang, X.-F., Liu, J.-N., Lu, L.-J., Peng, K.-M., Yang, G.-X., & Liu, J. (2016). Culture strategies for lipid production using acetic acid as sole carbon source by *Rhodosporidium toruloides*. *Bioresource Technology*, *206*, 141–149. https://doi.org/10.1016/j.biortech.2016.01.073

Jakobsen, A. N., Aasen, I. M., Josefsen, K. D., & Strøm, A. R. (2008). Accumulation of docosahexaenoic acid-rich lipid in thraustochytrid *Aurantiochytrium* sp. strain T66: Effects of N and P starvation and O$_2$ limitation. *Applied Microbiology and Biotechnology*, *80*(2), 297. https://doi.org/10.1007/s00253-008-1537-8

Karamerou, E. E., Theodoropoulos, C., & Webb, C. (2016). A biorefinery approach to microbial oil production from glycerol by *Rhodotorula glutinis*. *Biomass and Bioenergy*, *89*, 113–122. https://doi.org/10.1016/j.biombioe.2016.01.007

Laopaiboon, L., Phukoetphim, N., & Laopaiboon, P. (2016). Logistic function and modified Gompertz models for batch ethanol fermentation kinetics from sweet sorghum juice under high gravity condition. *New Biotechnology*, *33*, S86–S87. https://doi.org/10.1016/j.nbt.2016.06.1019

Li, Y., Zhao, Z. (Kent), & Bai, F. (2007). High-density cultivation of oleaginous yeast *Rhodosporidium toruloides* Y4 in fed-batch culture. *Enzyme and Microbial Technology*, *41*(3), 312–317. https://doi.org/10.1016/j.enzmictec.2007.02.008

Ma, X., Gao, Z., Gao, M., Ma, Y., Ma, H., Zhang, M., Liu, Y., & Wang, Q. (2018). Microbial lipid production from food waste saccharified liquid and the effects of compositions. *Energy Conversion and Management*, *172*, 306–315. https://doi.org/10.1016/j.enconman.2018.07.005

Marudkla, J., Lee, W.-C., Wannawilai, S., Chisti, Y., & Sirisansaneeyakul, S. (2018). Model of acetic acid-affected growth and poly(3-hydroxybutyrate) production by *Cupriavidus necator* DSM 545. *Journal of Biotechnology*, *268*, 12–20. https://doi.org/10.1016/j.jbiotec.2018.01.004

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*(3), 426–428. https://doi.org/10.1021/ac60147a030

Ong, H. C., Mahlia, T. M. I., Masjuki, H. H., & Norhasyima, R. S. (2011). Comparison of palm oil, Jatropha curcas and *Calophyllum inophyllum* for biodiesel: A review. *Renewable and Sustainable Energy Reviews*, *15*(8), 3501–3515. https://doi.org/10.1016/j.rser.2011.05.005

Phukoetphim, N., Salakkam, A., Laopaiboon, P., & Laopaiboon, L. (2017). Kinetic models for batch ethanol production from sweet sorghum juice under normal and high gravity fermentations: Logistic and modified Gompertz models. *Journal of Biotechnology*, *243*, 69–75. https://doi.org/10.1016/j.jbiotec.2016.12.012

Qasim, W. S., & Sultan, R. H. (2020). Production of single cell oil from local isolates *Rhodotorula glutinis*. *Journal of Microbiology, Biotechnology and Food Sciences*, *10*(2), 279–283. https://doi.org/10.15414/jmbfs.2020.10.2.279-283

Qin, L., Liu, L., Zeng, A.-P., & Wei, D. (2017). From low-cost substrates to single cell oils synthesized by oleaginous yeasts. *Bioresource Technology*, *245*, 1507–1519. https://doi.org/10.1016/j.biortech.2017.05.163

Ratledge, C., & Wynn, J. P. (2002). The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. *Advances in Applied Microbiology*, *51*, 1–51. https://doi.org/10.1016/s0065-2164(02)51000-5

Saenge, C., Cheirsilp, B., Suksaroge, T. T., & Bourtoom, T. (2011). Potential use of oleaginous red yeast *Rhodotorula glutinis* for the bioconversion of crude glycerol from biodiesel plant to lipids and carotenoids. *Process Biochemistry*, *46*(1), 210–218. https://doi.org/10.1016/j.procbio.2010.08.009

Satyanarayana, M., & Muraleedharan, C. (2011). A comparative study of vegetable oil methyl esters (biodiesels). *Energy*, *36*(4), 2129–2137. https://doi.org/10.1016/j.energy.2010.09.050

Soccol, C. R., Dalmas Neto, C. J., Soccol, V. T., Sydney, E. B., da Costa, E. S. F., Medeiros, A. B. P., & Vandenberghe, L. P. de S. (2017). Pilot scale biodiesel production from microbial oil of *Rhodosporidium toruloides* DEBB 5533 using sugarcane juice: Performance in diesel engine and preliminary economic study.

Bioresource Technology, *223*, 259–268. https://doi.org/10.1016/j.biortech.2016.10.055

Sutanto, S., Zullaikah, S., Tran-Nguyen, P. L., Ismadji, S., & Ju, Y.-H. (2018). *Lipomyces starkeyi*: Its current status as a potential oil producer. *Fuel Processing Technology*, *177*, 39–55. https://doi.org/10.1016/j.fuproc.2018.04.012

Tai, M., & Stephanopoulos, G. (2013). Engineering the push and pull of lipid biosynthesis in oleaginous yeast *Yarrowia lipolytica* for biofuel production. *Metabolic Engineering*, *15*, 1–9. https://doi.org/10.1016/j.ymben.2012.08.007

Wannawilai, S., & Sirisansaneeyakul, S. (2015). Economical production of xylitol from *Candida magnoliae* TISTR 5663 using sugarcane bagasse hydrolysate. *Kasetsart Journal - Natural Science*, *49*, 583–596.

Wu, S., Hu, C., Jin, G., Zhao, X., & Zhao, Z. K. (2010). Phosphate-limitation mediated lipid production by *Rhodosporidium toruloides*. *Bioresource Technology*, *101*(15), 6124–6129. https://doi.org/10.1016/j.biortech.2010.02.111

Wu, S., Zhao, X., Shen, H., Wang, Q., & Zhao, Z. K. (2011). Microbial lipid production by *Rhodosporidium toruloides* under sulfate-limited conditions. *Bioresource Technology*, *102*(2), 1803–1807. https://doi.org/10.1016/j.biortech.2010.09.033

Xue, F., Miao, J., Zhang, X., Luo, H., & Tan, T. (2008). Studies on lipid production by *Rhodotorula glutinis* fermentation using monosodium glutamate wastewater as culture medium. *Bioresource Technology*, *99*(13), 5923–5927. https://doi.org/10.1016/j.biortech.2007.04.046

Yang, J., Rasa, E., Tantayotai, P., Scow, K. M., Yuan, H., & Hristova, K. R. (2011). Mathematical model of *Chlorella minutissima* UTEX2341 growth and lipid production under photoheterotrophic fermentation conditions. *Bioresource Technology*, *102*(3), 3077–3082. https://doi.org/10.1016/j.biortech.2010.10.049

Yen, H.-W., & Liu, Y. X. (2014). Application of airlift bioreactor for the cultivation of aerobic oleaginous yeast *Rhodotorula glutinis* with different aeration rates. *Journal of Bioscience and Bioengineering*, *118*(2), 195–198. https://doi.org/10.1016/j.jbiosc.2014.01.002

Yen, H.-W., & Zhang, Z. (2011). Effects of dissolved oxygen level on cell growth and total lipid accumulation in the cultivation of *Rhodotorula glutinis*. *Journal of Bioscience and Bioengineering*, *112*(1), 71–74. https://doi.org/10.1016/j.jbiosc.2011.03.013

Zajšek, K., & Goršek, A. (2010). Modelling of batch kefir fermentation kinetics for ethanol production by mixed natural microflora. *Food and Bioproducts Processing*, *88*(1), 55–60. https://doi.org/10.1016/j.fbp.2009.09.002

Zhu, L. Y., Zong, M. H., & Wu, H. (2008). Efficient lipid production with *Trichosporon fermentans* and its use for biodiesel preparation. *Bioresource Technology*, *99*(16), 7881–7885. https://doi.org/10.1016/j.biortech.2008.02.033