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# OBTAINING GROWTH CURVES FOR *Scheffersomyces stipitis* STRAINS AND THEIR MODELING

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***Abstract***

*Growth curves are used in a wide range of applications such as crop science and biotechnology. Besides, mathematical models for fermentation can provide more information about kinetic of cell growth, and also promote the control and optimization of cell growth during fermentation. The main objectives of this study were undertaken not only plotted the cell growth curves, determined the specific growth equations, and calculated the kinetic parameters belong to Scheffersomyces stipitis strains (ATCC 58784 and 58785) but also to modelled their cell growths by using modified logistic and modified Richards models. The results indicated that the specific growth curves of ATCC 58784 in glucose and xylose mediums were $y=0.3047\times Abs_{600}-0.2656$ and $y=0.2322\times Abs_{600}+0.4329$, respectively. For ATCC 58785, they were $y=0.2639\times Abs_{600}+0.0282$ and $y=0.2323\times Abs_{600}+0.6211$, respectively. Furthermore, for ATCC 58784, maximum growth rate and doubling time values in glucose and xylose media were 0.23, 0.11 g/L/h and 2.45, 8.58 h, respectively. For ATCC 58785, they were 0.33, 0.11 g/L/h and 2.51, 7.66 h, respectively. In addition, modified logistic and modified Richards models were tested in order to describe cell growth profiles during fermentation by S. stipitis strains. Results indicated that these models can serve as a universal equation to fit cell growth. Moreover, validation of these models demonstrated that cell growth was all predicted accurately (slope=0.96 and 0.97, $R^2$=0.998 and 0.998 for ATCC 58784 in xylose media by modified logistic and modified Richards models, respectively; slope=1.01 and 1.01, $R^2$=0.995 and 0.995 for ATCC 58785 in xylose media by modified logistic and modified Richards models, respectively).*

***Key words:*** *cell growth curve, Scheffersomyces stipitis, modified logistic, modified Richards.*

## INTRODUCTION

In biotechnology, batch culture is a sealed system without any inlet or outlet streams since the nutrients are prepared in a volume-stable liquid media. Then the inoculum prepared under certain conditions for each microorganism is aseptically transferred in the media, which slowly grow and reproduce. Nutrients are exhausted and end products are formed as well as cell propagates. The biomass concentration is one of the major determinants to define the stage of cell growth, which is essential to understand the checkpoints of the cell growth. There are numerous methods to predict cell growth by direct or indirect measurements such as dry cell weight (DCW), optical cell density (OD), cell turbidity, cell respiration, metabolic rate, and metabolites, which are fairly favorable for analyzing cell growth. However, DCW and OD are the most used approaches to determine the microbial growth (Flickinger, 2013; Najafpour, 2015).

Kinetic modeling of value-added products' production by microbial fermentation is important since simplify the control and optimization of cell growth as well as product formation at different fermentation conditions such as pH, temperature, medium content, agitation, aeration, etc. To create kinetic model, cell growth has to be accounted and modeled. In general, cell growth indicates a phase containing lag, exponential, stationary, and death phases. In this phase, specific growth rate begins along with inoculation and maximum growth rate reaches to the highest value for a length of time, which leads to determine of lag time. Furthermore, while cell growth stops in stationary phase and $dx/dt$ is zero, in death phase, the growth rate reaches zero by decreasing, therefore an asymptote is reached. When the cell growth curve is described as the logarithm of cell number plotted versus time, which leads to a sigmoidal curve containing lag phase, exponential phase, and stationary phase as mentioned above (Zwietering et al., 1990).

In order to identify a cell growth curve and to decrease measured data, several cell growth models such as modified logistic and modified Richards are developed, which are describe not only the cell growth, but also to define the substrate consumption and product formation (Zwietering et al., 1990). In this paper, the cell growth curves for *S. stipitis* strains (ATCC 58784 and ATCC 58785) were obtained in glucose and xylose media and also some kinetic parameters related to cell growth were calculated. Besides, cell growth curves were modelled by using modified logistic and modified Richards models. However, the substrate level and product concentration are not of interest in our study since the objective of this study is also only to monitor the cell growth of *S. stipitis* strains.

## MATERIALS AND METHODS

### Microorganisms and mediums

The yeasts used to obtain cell growth curves were *Scheffersomyces stipitis* (formerly *Pichia stipitis*) strains ATCC 58784 and ATCC 58785, which were obtained from American Type Culture Collection (Manassas, VA, USA). *S. stipitis* ATCC 58784 was grown at 30ºC for 48 h in a yeast extract-malt (YM) medium containing 10 g of glucose, 3 g of yeast extract, 3 g of malt extract, and 5 g of peptone per liter of deionized water. The pH was adjusted to 6.2 with 4 N NaOH and HCl. *S. stipitis* ATCC 58785 was grown at 30ºC for 48 h in a yeast extract-peptone (YPD) medium containing 20 g of glucose, 10 g of yeast extract and 20 g of peptone per liter of deionized water. The medium pH was adjusted to 5.6 with 4 N NaOH and HCI. The cultures were stored at 4ºC and sub-cultured bi-monthly in order to maintain viability. For a long-term storage, stock cultures were maintained in 20% glycerol at -80ºC. *S. stipitis* strains were grown in 250 mL flasks containing 100 mL of YM or YPD at 30ºC and 150 rpm for 24 h for inoculation (Lee et al., 2011; Zhu et al., 2014).

### Fermentations

For cell growth curves, fermentations were carried out in a shaking incubator (CERTOMAT® IS, Goettingen, Germany) with 250 ml flasks containing 150 ml of YM and YPD. Temperature was maintained at 30ºC, agitation rate was set to 150 rpm, and 1% (v/v) of inoculum was used for fermentation. The pH of mediums were initially adjusted to 6.2 and 5.6 for the strain *S. stipitis* ATCC 58784 and ATCC 58785, respectively. Sampling (2 ml) was performed at every hour.

### DCW and OD

DCW in a fermentation broth was measured by drying in an oven. Firstly, one milliliter of fermentation broth was transferred into tared eppendorf tubes (total volume of 2 ml) and centrifuged at 14000 rpm for 10 min. Then supernatant was removed and the cells were washed twice with deionized water. Finally, the cells were dried at 85ºC to constant weight (Koch, 2013). The OD of the fermentation broth was measured using a spectrophotometer (ThermoScientific 201 UV-Visible Evolution, Shanghai, China) at 600 nm. Uninoculated fermentation broth was used as a blank (Lee et al., 2011; Zhu et al., 2011).

### Mathematical models

The modified logistic and modified Richards models were used to fit data related to cell growth curves.

#### *Modified logistic model*

The modified logistic function was used to describe the cell growth. Zwietering et al. (1990) modified the logistic equation (Pearl and Reed, 1920) to include parameters with biological meaning yielding the modified logistic equation.

$$X_t = \frac{X_m}{1 + \exp\left(\frac{4Q_X \times (\lambda - t) + 2 \times X_m}{X_m}\right)}$$

#### *Modified Richards model*

The modified Richards function was utilized to define the cell growth. Zwietering et al. (1990) modified the Richards equation (Richards, 1959) to involve parameters with biological meaning yielding the modified Richards equation. The symbols used in equation were given in Table 1.

$$X_t = X_m \times \left\{ 1 + v \times \exp(1+v) \times \exp\left( \frac{Q_X \times (1+v)^{\left(1+\frac{1}{v}\right)} \times (\lambda - t)}{X_m} \right) \right\}^{\left(\frac{-1}{v}\right)}$$

**Table 1.** The kinetic parameters belong to cell growth and mathematical models

| Kinetic parameters | *S. stipitis* ATCC 58784 | | *S. stipitis* ATCC 58785 | |
|---|---|---|---|---|
| | Glucose media | Xylose media | Glucose media | Xylose media |
| Biomass production (*X*, g/L) | 4.10 | 3.86 | 4.30 | 3.49 |
| Maximum biomass concentration ($X_{max}$, g/L) | 4.21 | 4.38 | 4.47 | 4.19 |
| Maximum growth rate ($Q_X$, g/L/h) | 0.23 | 0.11 | 0.33 | 0.11 |
| Shape parameter for modified Richards model (*v*) | 0.28 | 0.08 | 0.28 | 0.09 |
| Doubling time ($t_d$, h) | 2.45 | 8.58 | 2.51 | 7.66 |
| Lag time (λ, h) | 3.90 | 0.50 | 2.80 | 0.00 |

The shape parameter (*v*) was calculated with non-linear least squares regression procedure in order to the least error value and the nearest $R^2$ value to 1.

## RESULTS AND DISCUSSIONS

The current study was designed not only to obtain the cell growth curves for *S. stipitis* strains using different medium compositions in point of carbon sources and to calculate some kinetic parameters such as $Q_X$ and $t_d$ but also to model mathematically the obtained growth curves by using modified logistic and modified Richards models.

### Determination of cell growth curves for *S. stipitis* strains

The growth curves in terms of absorbans and weight values were initially plotted versus time. Then, specific growth equations for *S. stipitis* strains were obtained with weighed weight values plotted versus the OD values at the same times. $R^2$ values and the specific cell growth equations were achieved along with the addition of trendlines (Madigan, 2005).

### *Scheffersomyces stipitis* ATCC 58784

The growth curves for *S. stipitis* ATCC 58784 by using both glucose and xylose mediums were obtained as can be seen in Figure 1. According to results, in glucose medium (Figure 1A-B), cell growth equation was found to be $y=0.3047\times Abs_{600}-0.2656$, where *y* is cell concentration (g/L), while $R^2$ value was calculated as 0.9985. In xylose medium (Figure 1C-D), cell growth equation was obtained to be $y=0.2322\times Abs_{600}+0.4329$, where *y* is cell concentration (g/L), while $R^2$ value was estimated to be 0.9960. Therefore, these equations were completely represented the growth curves of *S. stipitis* ATCC 58784. On the other hand, the kinetic parameters with related to cell growth such as *X*, $X_{max}$, $Q_X$, $t_d$, and λ were also computed, as can be seen in Table 1. In glucose medium, the kinetic parameters were determined as 4.10 g/L, 4.21 g/L, 0.23 g/L/h, 2.45 h, and 3.9 h, respectively. Furthermore, in xylose medium, they were found as 3.86 g/L, 4.38 g/L, 0.11 g/L/h, 8.58 h, and 0.5 h, respectively. In conclusion, *S. stipitis* ATCC 58784 was successfully grown in both mediums, but its $t_d$ value was too long in xylose medium compared to glucose media due to lower specific growth rate, while the lag time was too short in xylose medium compared to glucose media.

### *Scheffersomyces stipitis* ATCC 58785

The growth curves for *S. stipitis* ATCC 58785 by using both glucose and xylose mediums were obtained as can be seen in Figure 2. According to results, in glucose medium (Figure 2A-B), specific growth equation was found to be $y=0.2639\times Abs_{600}+0.0282$, where *y* is cell concentration (g/L), while $R^2$ value was calculated as 0.9954. In xylose medium (Figure 2C-D), specific growth equation was obtained to be $y=0.2323\times Abs_{600}+0.6211$, where *y* is cell concentration (g/L), while $R^2$ value was estimated to be 0.9946. Therefore, these equations were completely represented the growth curves of *S. stipitis* ATCC 58785. On the other hand, the kinetic parameters with related to cell growth as above were also computed, as can be seen in Table 1.

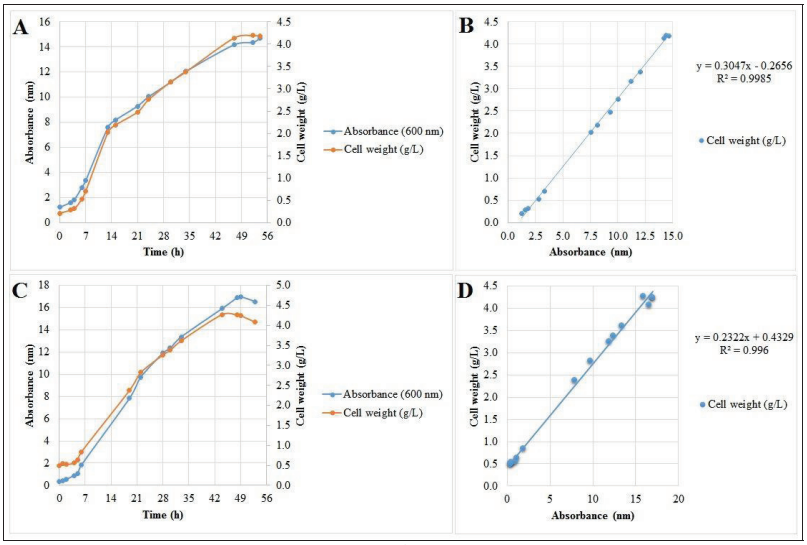


***Figure 1. A:*** *Cell growth curve in point of absorbance and cell weight for* S. stipitis *ATCC 58784 in glucose medium,* ***B:*** *Specific growth equation for* S. stipitis *ATCC 58784 in glucose medium,* ***C:*** *Cell growth curve in point of absorbance and cell weight for* S. stipitis *ATCC 58784 in xylose medium,* ***D:*** *Specific growth equation for* S. stipitis *ATCC 58784 in xylose medium.*

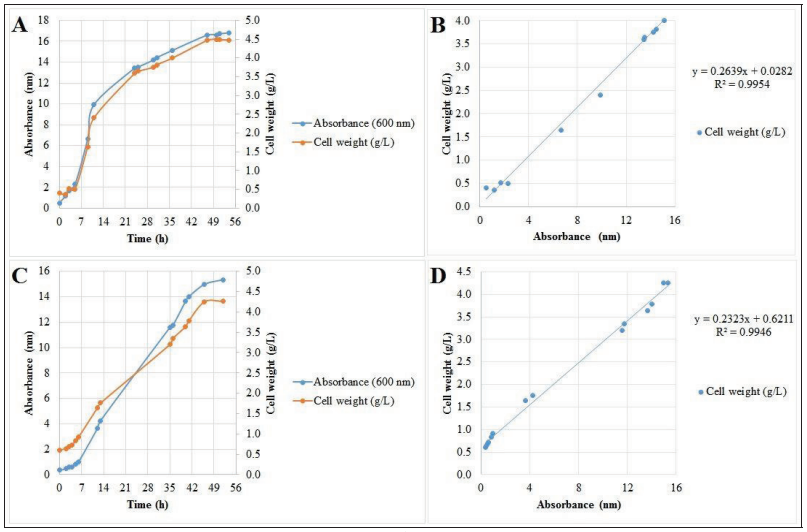


***Figure 2. A:*** *Cell growth curve in point of absorbance and cell weight for S. stipitis ATCC 58785 in glucose medium,* ***B:*** *Specific growth equation for S. stipitis ATCC 58785 in glucose medium,* ***C:*** *Cell growth curve in point of absorbance and cell weight for S. stipitis ATCC 58785 in xylose medium,* ***D:*** *Specific growth equation for S. stipitis ATCC 58785 in xylose medium.*

In glucose medium, the kinetic parameters were determined as 4.10 g/L, 4.21 g/L, 0.23 g/L/h, 2.45 h, and 3.9 h, respectively. Furthermore, in xylose medium, they were found as 3.86 g/L, 4.38 g/L, 0.11 g/L/h, 8.58 h, and 0 h, respectively. Consequently, *S. stipitis* ATCC 58785 was successfully grown in both mediums, but its $t_d$ value was too long in xylose medium compared to glucose media due to lower specific growth rate, while the lag time was too short in xylose medium compared to glucose media.

**Modeling of cell growth**

The kinetic parameters obtained from fermentations (Table 1) were applied to

modified logistic and modified Richards equations for cell growth. Afterwards, an independent set of fermentation date was utilized to validate the constructed model. The modified logistic and modified Richards equations were applied to fit cell growth. Subjective comparisons of the actual cell growth curve with modified logistic and modified Richards models were carried out by plotting both the experimental and the predicted values obtained from the models (Figure 3A-D). The modified logistic and modified Richards models sufficiently fitted the experimental data of all cell growth curves. Further validation was obtained via regression thorough origin of experimental data and predicted values obtained via modified logistic and modified Richards models. Results were shown in Table 2.

For *S. stipitis* ATCC 58784, $R^2$ values in glucose and xylose mediums were 0.954 and 0.998 and slops were 1.10 and 0.96 by using modified logistic model, respectively. In addition, $R^2$ values of glucose and xylose mediums were 0.963 and 0.998 and slopes were 1.13 and 0.97 by using modified Richards model, respectively (Table 2). The results demonstrated that the modified logistic and modified Richards models can sufficiently define the experimental data (Figure 3). The values of RMSE in glucose and xylose mediums were 0.53 and 0.11 g/L by using modified logistic model while they were 0.47 and 0.22 g/L by using modified Richards model, respectively. Besides, the values of MAE in glucose and xylose mediums were 0.38 and 0.09 g/L by using modified logistic model while they were 0.31 and 0.19 g/L by using modified Richards model, respectively (Table 2). Results showing that the usage of modified logistic and modified Richards models can obviously represent the cell growth curves of *S. stipitis* ATCC 58784 in glucose and xylose mediums.

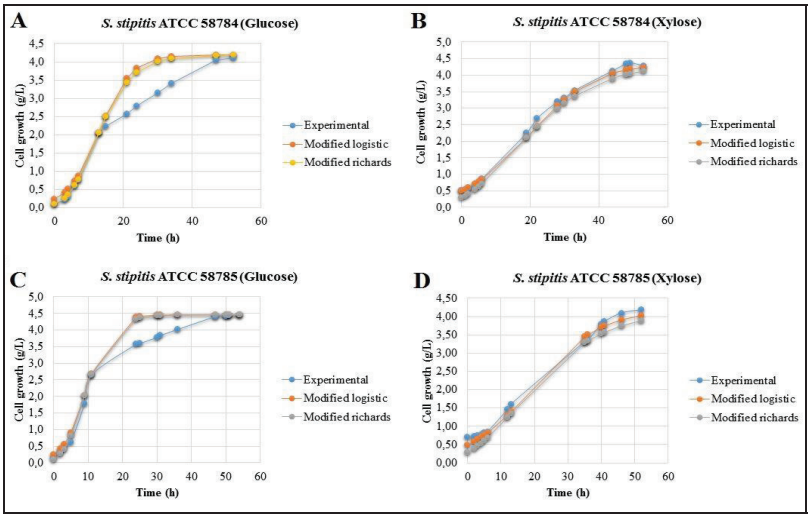


***Figure 3.*** *Experimental and predicted cell growth profiles by S. stipitis strains.*

**Table 2.** Validation of models for biomass production.

| Model | Microorganism and media | RMSE (g/L) | MAE (g/L) | $R^2$ | Slope |
|---|---|---|---|---|---|
| Modified logistic | *S. stipitis* ATCC 58784 (Glucose media) | 0.53 | 0.38 | 0.954 | 1.10 |
| | *S. stipitis* ATCC 58784 (Xylose media) | 0.11 | 0.09 | 0.998 | 0.96 |
| | *S. stipitis* ATCC 58785 (Glucose media) | 0.41 | 0.29 | 0.972 | 1.04 |
| | *S. stipitis* ATCC 58785 (Xylose media) | 0.14 | 0.13 | 0.995 | 1.01 |
| Modified Richards | *S. stipitis* ATCC 58784 (Glucose media) | 0.47 | 0.31 | 0.963 | 1.13 |
| | *S. stipitis* ATCC 58784 (Xylose media) | 0.22 | 0.19 | 0.998 | 0.97 |
| | *S. stipitis* ATCC 58785 (Glucose media) | 0.42 | 0.27 | 0.975 | 1.08 |
| | *S. stipitis* ATCC 58785 (Xylose media) | 0.26 | 0.24 | 0.995 | 1.01 |

**RMSE:** Root-mean-square errors. **MAE:** Mean absolute error.

For *S. stipitis* ATCC 58785, while $R^2$ values in glucose and xylose mediums were 0.972 and 0.995 and slops were 1.04 and 1.01 by using modified logistic model, they were 0.975 and 0.995 and slopes were 1.08 and 1.01 by using modified Richards model, respectively (Table 2). The results demonstrated that the modified logistic and modified Richards models can adequately define the data (Figure 3). The values of RMSE in glucose and xylose mediums were 0.41 and 0.14 g/L by using modified logistic model while they were 0.42 and 0.26 g/L by using modified Richards model, respectively. On the other hand, while the values of MAE in glucose and xylose mediums were 0.29 and 0.13 g/L by using modified logistic model, they were 0.27 and 0.24 g/L by using modified Richards model, respectively (Table 2). Results indicating that the usage of modified logistic and Richards models can evidently represent the cell growth curves of *S. stipitis* ATCC 58785 in glucose and xylose mediums (Figure 3).

## CONCLUSIONS

In this study, the cell growth curves belong to *S. stipitis* strains (ATCC 58784 and ATCC 58785) were determined and their specific growth equations were obtained. In addition, the kinetic parameters belong to cell growth of *S. stipitis* strains were also calculated. Besides, modeling of fermentations towards cell growth of *S. stipitis* strains in media was also investigated and achieved. According to results, the specific growth equation of ATCC 58784 in glucose medium was $y=0.3047\times Abs_{600}-0.2656$. In xylose medium, it was $y=0.2322\times Abs_{600}+0.4329$. For ATCC 58785, the specific growth equation was $y=0.2639\times Abs_{600}+0.0282$ in glucose media while it was $y=0.2323\times Abs_{600}+0.6211$ in xylose medium. Moreover, for *S. stipitis* ATCC 58784, $Q_X$ and $t_d$ values in glucose and xylose media were 0.23, 0.11 g/L/h and 2.45, 8.58 h, respectively. For *S. stipitis* ATCC 58785, they were 0.33, 0.11 g/L/h and 2.51, 7.66 h, respectively. We also modelled the cell growth curves of *S. stipitis* strains by using modified logistic and modified Richards models. Modified logistic and modified Richards models in glucose and xylose mediums (slop=1.10 and 1.13, $R^2$=0.954 and 0.963; slop=0.96 and 0.97, $R^2$=0.998 and 0.998, respectively) adequately described the cell growth of *S. stipitis* ATCC 58784. Also they were accurately defined the cell growth of *S. stipitis* ATCC 58785 (slop=1.04 and 1.08, $R^2$=0.972 and 0.975; slop=1.01 and 1.01, $R^2$=0.995 and 0.995, respectively). Consequently, the specific growth equations-obtained for *S. stipitis* strains in glucose and xylose mediums can be used every time to determine the cell growth. In addition, the modified logistic and modified Richards equations proposed in this study indicated its generality to fit all cell growth curves.

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