# Budding yeast colony growth study based on circular granular cell

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**Abstract.** Yeast colony growth can be modelled by using circular granular cells, which can grow and produce buds. The bud growth angle can be set to regulate cell budding pattern. Cohesion force, contact force and Stokes force were adopted to accommodate the behaviour and interactions among cells. Simulation steps are divided into two steps, the explicit step is due to cell growing and implicit step for the cell rearrangement. Only in explicit step that time change was performed. In this study, we examine the influence of cell diameter growth time and reproduction time combination toward the growth of cell number and colony formation. We find a commutative relation between the cell diameter growth time and reproduction time to the specific growth rate. The greater value of the multiplication of the parameters, the smaller specific growth rate is obtained. It also shows a linear correlation between the specific growth rate and colony diameter growth rate.

## 1. Introduction

A multicellular behaviour that exhibited by budding yeast cells is biofilm formation. In pathogenic yeast such as *Candida sp*, biofilm structure is associated with fungal infection in the human body [1]. Other species of budding yeast, *Saccharomycess cerevisiae,* is well known as non-pathogenic budding yeast that has similar characteristic in forming colony structure with *Candida sp*. Thus, biofilm formation in *Saccharomycess cerevisiae* becomes a model for fungal biofilm formation [2].

This complex structure starts with colony formation in growth media. Observation of real colony formation was carried out through a video [3]. In this work, simulation is performed to mimic the colony formation. The initial configuration of the colony in simulation is set similar to the initial configuration of the colony from video (figure 1). Colony growth is assumed to be seen above. Theoretical observation was also done to ensure that simulation results as it should be in biological system.

## 2. Budding Yeast Colony Growth

*Saccharaomycess cerevisiae* cell is in ellipsoidal shape with the diameter between 5 and 10 micrometers. This species reproduces asexually by forming bud on the surface of precursor cell. The reproduction is occurring asymmetrically. Nucleus division has happened when daughter cell size is smaller than its mother cell. After the division is completed, daughter cell size keeps increasing until it reaches its maximum size and ready to reproduce.

Colony formation is found in semisolid media such as agar. If nutrient is sufficient, the growth of cell number is described by following equation [4]

$$\frac{dN}{dt} = \lambda t \quad . \tag{1}$$

Integration of the equation gives

$$\ln N = \lambda t + \ln N_0 \, , \tag{2}$$

where $N$ is the number of cells that present at time t, $N_0$ is the number of cells at $t$ = 0 and $\lambda$ is cell number growth rate or specific growth rate. In nutritious growth media, colony can be found in circular shape [5]. At the initial stage of growth, when the colony diameter is less than 0.2 mm or about 20 cells in cell diameter unit by assuming a cell diameter is 10 micrometers, colony diameter is observed increase exponentially [4].

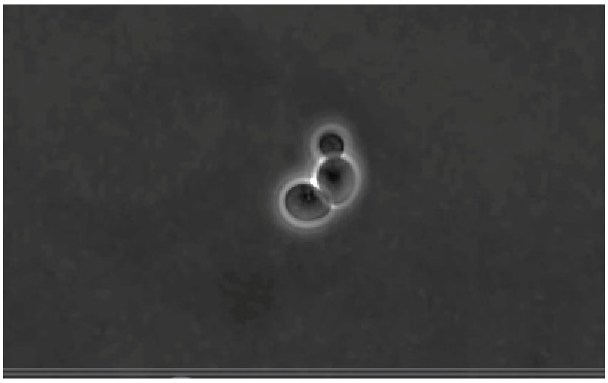


**Figure 1.**Initial growth of budding yeast colony under fluorescent microscope from video experiment [3].

## 3. Cell Growth Modelling

Cell growth is included cell diameter growth and cell division (reproduction). The cell is modelled by using granular cell in circular shape with diameter $d_i$ [7]. Cells diameter growth is defined as follows

$$d_i(t) = \begin{cases} 0, & t < t_{i0}, \\ d_i(t - \delta t) + v_D \delta t, & t_{i0} \le t \le t_G, \\ d_{\max} & t_G < t, \end{cases} \tag{3}$$

with $t_{i0}$, $v_D$, $d_{\max}$ and $t_G$ stand for cell birth time, diameter growth rate, maximum diameter, and diameter growth time, respectively. Diameter growth time $t_G$ is the time for cell to grow from bud initiation until reaching a maximum cell diameter. All cells have the same maximum cell diameter value.

At particular time $t$, a cell $i$ is located at $\vec{r}_i(t)$ correspond to the center of mass of the cell. "daughter" cell emerges at a position in "mother" cell circumference with direction or bud angle $\theta$ (figure 2(a)). Every mother cell can reproduce only one daughter cell in a reproduction time. The period between two daughter cell initiation at a mother cell is termed as $t_B$. Cell growth time is illustrated in figure 2(b).

Movement of cells is governed by three forces, namely cohesion force, contact force, and Stokes force. The first two forces are to accommodate the interaction of cells. Cohesion force is adapted from gravitation force to mimic the attractive interaction between cells, while contact force is repulsive force that is happening because of overlap between two cells. The third force, Stoke forces, represents the friction between cell and growth media.

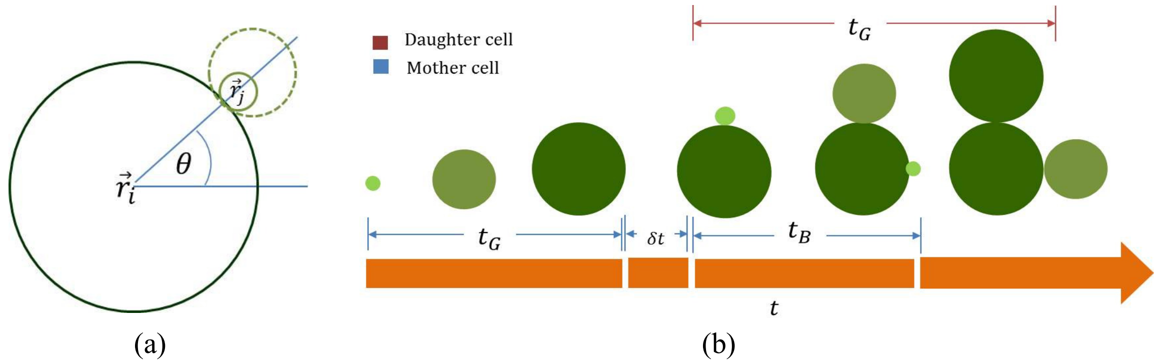



**FIGURE 2.** (a) Daughter cell (bud) emerges in $\theta_j = \frac{1}{4}\pi$ at mother cell. (b) Cell growth times are governed by diameter growth time $t_G$ and reproduction time $t_B$.

Cohesion force between two cells is defined as follows

$$\vec{F}_{ij}^{\text{coh}} = -G\frac{m_i m_j}{r_{ij}^{\ 2}}\hat{e}_{ij}\,, \tag{4}$$

where $r_{ij}$ is the distance between two cells, and $G$ represents the strength of cohesion between cells. Contact force is affected by the overlap between two cells $\xi_{ij}$ , which is defined as

$$\xi_{ij} = \max\left(0, R_i + R_j - r_{ij}\right), \tag{5}$$

with $R_i$ and $R_j$ represent radius of cell $i$ and $j$. The contact or collision force is defined as follows

$$\vec{F}_{ij}^{\text{col}} = \left(k_r \xi_{ij} - k_v v_{ij}\right)\hat{e}_{ij}\,. \tag{6}$$

The term of $k_r$ and $k_v$ is spring coefficient and damping coefficient, respectively. Stokes force for spherical object that moving through fluid in low speed is adapted to represent cell movement in growth media, defined as

$$\vec{F}_i^{\text{flu}} = -6\pi\eta R_i \vec{v}_i\ , \tag{7}$$

which $\eta$ is termed as friction coefficient that represent the viscosity of growth media.

From (4), (6), and (7), the total force that suffered by $i$-th cell $\vec{F}_i$ is obtained. Using Newton's second law, acceleration of every cell is calculated every time $t$. Then, from the acquired acceleration, velocity and position of every cell is determined by Euler integration method.

## 4. Simulation

Simulation steps are divided into two step, explicit step and implicit step. The explicit step is due to cell growing and implicit step for the cell rearrangement. Only in explicit step that time change was performed.

Simulation is restricted by cell maximum number $N_{\max}$. Initial configuration of colony consists of three cells which are initially at $\vec{r}_1$ = (0, 0), $\vec{r}_2$ = (-1.0, -1.0), and $\vec{r}_3$ = (0.1, 0.6) to mimic the initial configuration of cells colony in video. Other parameters that are used in this simulation are presented in table 1.

**TABLE 1** Simulation parameters.

| Parameters | Value |
|---|---|
| $N_{max}$ | 50 |
| $d_{max}$ | 1.0 |
| $k_r$ | $10^4$ |
| $k_v$ | 1.0 |
| $G$ | 5.0 |
| $\eta$ | 15.0 |
| $\Delta t$ | $10^{-5}$ |
| $m_i$ | 1.0 |
| Implicit iteration | 5 |
| $t_B$ | 3, 3.2, 3.5, and 3.8 |
| $t_G$ | 3, 3.2, 3.5, and 3.8 |

All simulation parameters are chosen deliberately. The value of diameter growth time $t_G$ and reproduction time $t_B$ are combined. A combination of $t_B$ and $t_G$ value is used for one simulation. There are 16 combinations of diameter growth time and reproduction time that used in simulation. The bud initial angle that is used in the simulation is $\theta = \frac{1}{4}\pi$ where $n$ is an integer number that chosen randomly between 0, 1, 2 and 3.

## 5. Result and Discussion

The influence of a combination of diameter growth time and reproduction time is shown by the growth of cell number in the colony and its diameter growth. Both of them are observed from $t$ = 0 until $t$ = 12. Simulation results are compared to the real colony growth from video.

### *5.1 Growth of cell number*

Figure 3 shows simulation results for combination value $t_B = 3$ and $t_G = 3$ and colony growth from video at $t$ = 12. To obtain cell number growth rate, cell number for every time $t$ both of simulations and real colony are calculated. Then, the data are fitted by simple linear function as follows

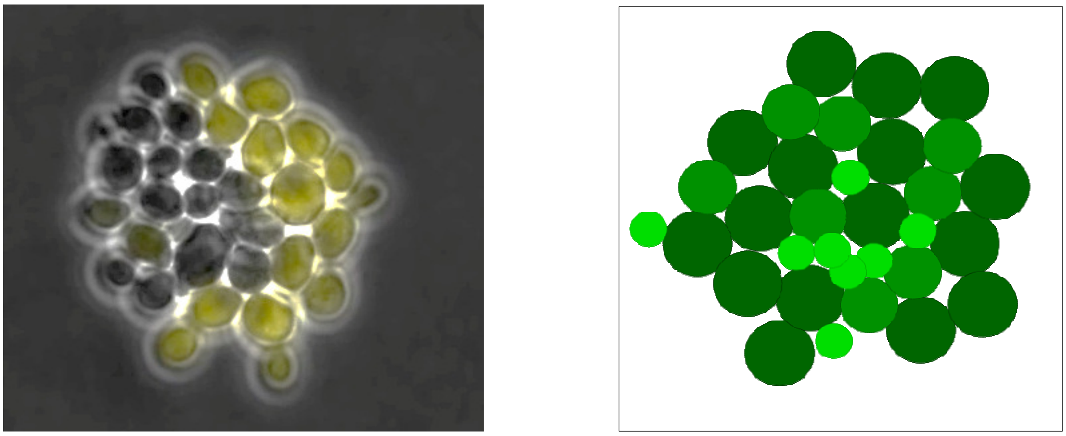
$$y = ax + b \; . \tag{8}$$

The term $a$ in (8) refers to the cell number growth rate or specific growth rate λ in (2), while $b$ refers to natural logarithm of initial cell number $N_0$. Cell number growth rate $\lambda$ for some simulation is presented in figure 4.

The specific growth rate value of all simulations is presented in table 2. It shows that the greater multiplication value of $t_B$ and $t_G$, the smaller specific growth rate $\lambda$ is obtained. For longer diameter growth time and reproduction time, the smaller number of cells in colony is achieved. Hence, there is commutative relation between the cell diameter growth time and reproduction time to the specific growth rate. The same multiplication result of diameter growth time $t_G$ and reproduction time $t_B$ gives same specific growth rate value. Specific growth rate values $\lambda$ of simulation with following $t_B$ and $t_G$ combination value is smaller from the specific growth rate of real colony growth.

### *5.2 Growth of colony diameter*

Colony diameter $D$ measurement is determined by the minimum area of a circle that can cover all cells in the colony [6]. The measurement is carried out by minimizing the empty space that covered by the circle. The colony diameter is measured in cell diameter unit. Colony diameter growth rates that resulted in simulation are less than 20 cells in cell diameter unit [7]. Therefore the growth of the colony should increase exponentially in accordance with the theory [4].



(a) (b)

**FIGURE 3**. Real colony formation at $t = 12$ (left) and simulation result with $t_B = 3$, $t_G = 3$ (right). In simulation result, darker color indicates older cell.

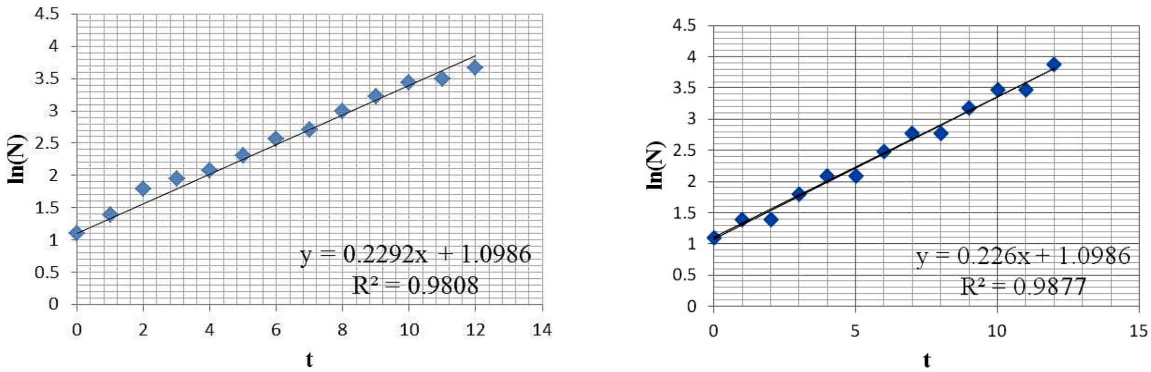



**FIGURE 4.** The specific growth rate of real colony is $\lambda_c = 0.2292$ (left) and in simulation using parameters $t_B = 3$ and $t_G = 3$ is $\lambda = 0.226$ (right).

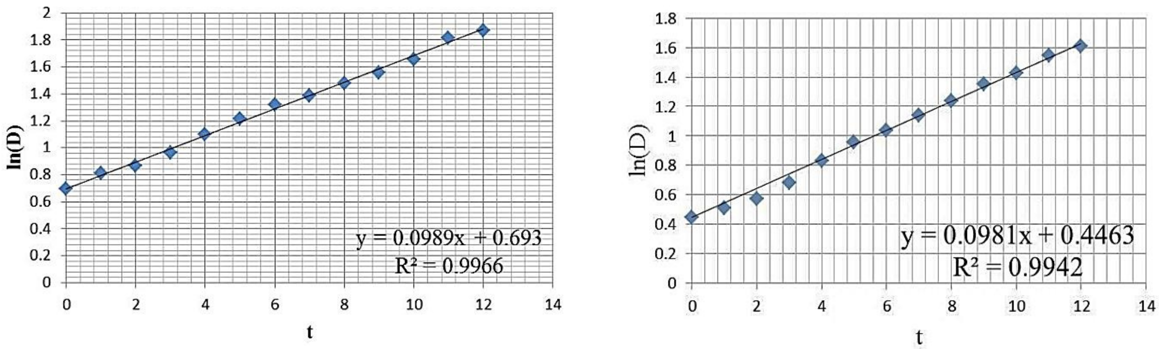



**FIGURE 5.** Colony diameter growth rate of real colony $\mu_c = 0.989$ (left) and simulation result with $t_B = 3.5$ and $t_G = 3.2$, $\mu = 0.0981$ (right).

**TABLE 2.** Specific growth rate and colony diameter growth rate value of all simulation.

| $t_B$ | $t_G$ | $\lambda$ | $\lvert\lambda - \lambda_c\rvert$ | $\mu$ | $\lvert\mu - \mu_c\rvert$ |
|---|---|---|---|---|---|
| 3 | 3 | 0.226 | 0.0032 | 0.1084 | 0.0095 |
| 3 | 3.2 | 0.2256 | 0.0036 | 0.1048 | 0.0059 |
| 3 | 3.5 | 0.2151 | 0.0141 | 0.1008 | 0.0019 |
| 3 | 3.8 | 0.2028 | 0.0264 | 0.0951 | 0.0038 |
| 3.2 | 3 | 0.2256 | 0.0036 | 0.1044 | 0.0055 |
| 3.2 | 3.2 | 0.2185 | 0.0107 | 0.1026 | 0.0037 |
| 3.2 | 3.5 | 0.206 | 0.0232 | 0.0965 | 0.0024 |
| 3.2 | 3.8 | 0.1984 | 0.0308 | 0.0946 | 0.0043 |
| 3.5 | 3 | 0.2151 | 0.0141 | 0.1046 | 0.0057 |
| 3.5 | 3.2 | 0.206 | 0.0232 | 0.0981 | 0.0008 |
| 3.5 | 3.5 | 0.2016 | 0.0276 | 0.0936 | 0.0053 |
| 3.5 | 3.8 | 0.1905 | 0.0387 | 0.09 | 0.0089 |
| 3.8 | 3 | 0.2028 | 0.0264 | 0.0931 | 0.0058 |
| 3.8 | 3.2 | 0.1984 | 0.0308 | 0.0943 | 0.0046 |
| 3.8 | 3.5 | 0.1905 | 0.0387 | 0.0928 | 0.0061 |
| 3.8 | 3.8 | 0.1869 | 0.0423 | 0.0872 | 0.0117 |

The result of colony diameter measurement is fitted by using the same linear function as in (8) Term $a$ and $b$ in figures 5(a) and 5(b) refer to colony diameter growth rate $\mu$ and the natural logarithm of initial colony diameter, respectively. All colony diameter growth rates $\mu$ are also presented in table 2. Colony diameter growth rate $\mu$ of real colony is close to colony diameter growth rate in simulation with parameters $t_B = 3.5$ and $t_G = 3.2$.

The commutative relation between diameter growth time and reproduction time is not exhibited in colony diameter growth rate. This is due to different cell compaction process in colony formation. The correlation between cell number growth rate $\lambda$ and colony diameter growth rate $\mu$ is presented in following figure 6.

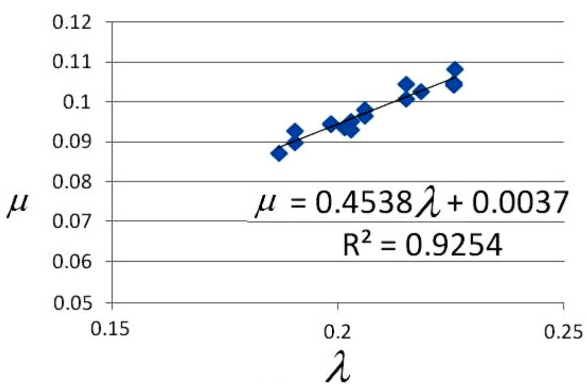



**FIGURE 6.** Linear correlation is presented between colony diameter growth rate $\mu$ and cell number growth rate $\lambda$.

## 6. Conclusion
Budding yeast colony growth can be modeled by circular granular cell. For colony diameter less than twenty cells in cell diameter unit, the growth of cell number and colony diameter can exhibit exponential growth. By choosing combination value of diameter growth time $t_G$ and reproduction time $t_B$ between 3,3.2,3.5 and 3.8, it was found that there is a commutative relation between the combination of parameter value in specific growth rate but not in colony diameter growth due to the compaction process. The greater value of the multiplication of diameter growth time and reproduction time, smaller specific growth rate is obtained. It also shows a linear correlation between specific growth rate and colony diameter growth rate.

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