Dynamic Study of *Tetrahymena pyriformis* Growth and Reproduction in Aerobic and Anaerobic Conditions

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ABSTRACT : The population growth and reproduction of *Tetrahymena pyriformis* were studied under shaken (aerobic) and unshaken (anaerobic) conditions by applying the growth models, exponential and logistic growth models and the population growth of *Tetrahymena* was showed the logistic growth model under both, shaken and unshaken conditions and also, the more oxygenated samples had greater population size (N) and three times faster growth rate (r) than less oxygenated samples during incubation periods.

Key words : *Tetrahymena pyriformis*, Exponential, Logistic, Growth models, Aerobic, Anaerobic.

INTRODUCTION

There are two most important models of population growth, which are the exponential and the logistic growth models (Campbell & Reece, 2007; Frankel et al., 2007). The exponential model is based on unlimited reproduction of organisms, whereas the logistic model combines reproduction and competition processes. The exponential model, introduced by Thomas R. Malthus, represents that population increase by exponentially under ideal conditions with abundant resources and without any limiting factors (Begon et al., 2006). The exponential growth is defined by $\frac{dN}{dt} = (b-d)N = r \cdot N$ where $\frac{dN}{dt}$ is the instantaneous population growth rate, b is per capita birth rate, d is per capita death rate, N is population size, and r is per capita growth rate. In the case of r that is greater than zero, b is greater than d, population increases at faster rate, and J-shaped curve is shown on the plot of population size (N on y-axis) versus time (t on x-axis) using arithmetic graph paper. A straight line is also shown on the plot of N versus time (t) using semi-logarithmic graph paper that is reduced by ten folds scale on y-axis.

In the case of growth rate (r) that is smaller than zero (r<0), population declines exponentially, and if r is equal to zero (r=0), population does not change. In the exponential growth, it is assumed that the r value is constant, but it is not realistic for many situations. As populations grow, intraspecific competition for limited resources appears, then the death rate (d) may be increased and the birth rate (b) may be decreased. Therefore, the growth rate (r) should decrease as the population size (N) increases. In the initial phase of growth, population can be growing rapidly according to exponential growth due to no limiting factors. In other words, exponential growth does occur in the short term, but does not continue in the long term.

The logistic model was developed by Pierre Verhulst in 1838, who suggested that the population growth rate might be limited because of population density (Begon et al., 2006). The logistic model is defined by $\frac{dN}{dt} = r \cdot N\left(1 - \frac{N}{k}\right)$ where r is per capita growth rate that declines as population size (N) increases. It is determined by the reproductive abilities of species. The r value of *Tetrahymena* would be much higher than that of human because *Tetrahymena* can reproduce faster than human. The logis-

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tic growth model shows S-shaped (sigmoidal) curve on the graph of population size (N) versus time (t) on the arithmetic graph paper (Odum, 1997; Begon et al., 2006). The rectangular hyperbola curve is also shown on the plot of N versus time (t) using semi-logarithmic graph paper. For small populations, the growth rate (r) value is positive and small population increases until the point where r is zero (r=0). In other words, population increases and reaches a plateau. It can be shown mathematically as follows. In the equation for logistic growth model, $\left(1-\dfrac{N}{k}\right)$ is the modifying factor. For a small population, N is much less than k (N≪k), then $\dfrac{N}{k}$ is a very small number and $\left(1-\dfrac{N}{k}\right)$ is almost equal to 1. When $\left(1-\dfrac{N}{k}\right)$ is close to 1, $\dfrac{dN}{dt}=r \cdot N\left(1-\dfrac{n}{k}\right)\approx r \cdot N$. The equation for this logistic growth is equal to the equation for exponential growth for a very small population. When a small population has grown to become a large population, then $\left(1-\dfrac{N}{k}\right)$ becomes a smaller number. The growth rate (r) decreases. Therefore, the large population declines in size until the point where r=0, that is, population decreases and reaches a plateau. The point where r value is zero (r=0) is an equilibrium point, that is, populations do not increase or decrease at this point. This point is called carrying capacity (k). The carrying capacity (k) represents the upper limit of population growth, the maximum numbers of individuals that the resources in the environment can maintain over time. In the logistic equation, $\dfrac{dN}{dt}=r \cdot N\left(1-\dfrac{n}{k}\right)=N\left(r-\dfrac{r}{k}\right)$, the coefficient r corresponds to reproduction and the coefficient $\dfrac{r}{k}$ corresponds to competition.

Tetrahymena pyriformis is a relatively large (length 40-60 *μ*m), unicellular ciliated protozoan (Dias, 2003; Collins, 2005). *Tetrahymena* reproduces asexually by binary fission in which each parent cell is divided into two daughter cells (Elliott et al., 1953; Virtue & Cole, 1999; Bruns et al., 2000; Lee et al., 2000). It lives in freshwater at optimum temperature 4℃ to 30℃ and optimum pH 7.25-7.30 (Numata et al., 2000; Kim et al., 2002). It can grow in aerobic and anaerobic conditions, however it has a greater reproduction rate in aerobic condition than in anaerobic condition. *Tetrahymena pyriformis* was used in this experiment due to its fast growth rate under common culture conditions (Niculescu, 2000; Williams, 2004). This experiment is studied to observe what the differences of population growth and reproduction of *Tetrahymena* in shaken and unshaken conditions are, what kinds of population growth model, exponential or logistic model, can be applied to each case, and how the limiting factors are affected to the carrying capacity of *Tetrahymena* growth and reproduction in both conditions. The population growth of *Tetrahymena* in aerobic and anaerobic conditions will show logistic growth model included the carrying capacity.

METHODS

The strains of *Tetrahymena* used in this experiment was obtained from the stock center of Cornell univ. The proteose and yeast extract and other materials were purchased from Sigma co. Before the experiment, two large culture flasks were prepared with a medium of proteose pepton and yeast extract, and then sterilized. Each flask was inoculated with a same numbers of *Tetrahymena*. They were grown at 28℃, an optimum temperature for *Tetrahymena*. Then one flask was shaken in order to promote oxygen supplement to the medium (aerobic sample). The other flask was unshaken in order to reduce the availability of oxygen (anaerobic sample). The samples were removed from the cultures at different time points: nine shaken flasks (after 0, 3, 6, 9, 12, 15, 24, 36, and 48 hours of incubation) and three unshaken flasks (after 9, 15, and 36 hours of incubation) were prepared. Then these samples were immediately killed and fixed by a chemical preservative to stop further hydrolytic degradation. To study the pattern of the population growth of *Tetrahymena*, the cells in each flask were counted using sub-sample instead of

main sample. The sub-sample is a confidently representative of the large sample. To prepare the sub-samples, twelve test tubes were prepared to transfer sample from the cultures as follows. Each test tube was mixed without introducing bubble which will interfere with counting. Three individuals were counted the cells in the twelve samples flasks twice in order to obtain accurate data. Using pipetman (Gilson), 100 ㎕ of well-mixed culture was transferred to the center of glass slide, and then a coverslip was placed on top of the slide. For test tubes of 9, 12, and 15 hours incubation (shaken sample), ten times dilutions were performed by addition of 9 ㎖ of plain water and 1 ㎖ of *Tetrahymena* sample. Twenty times dilutions were performed by addition of 19 ㎖ of plain water and 1 ㎖ of *Tetrahymena* sample in test tubes of 24, 36, and 48 hours incubation (shaken sample). With 10× objective lens of compound microscope, the numbers of cells were counted in divided field. Once the experimenters counted

cells in one field ("count per field"), I then moved on to the next field and counted that one. In order to obtain a "corrected count per field," samples diluted ten and twenty folds were multiplied by ten and twenty, respectively. The duplicate counts of shaken and unshaken samples from each time were averaged, combined, and averaged with the entire section's data. Then the data was plotted on arithmetic graph paper and semi-logarithmic paper.

RESULTS

Table 1 and 2 show the average population numbers of *Tetrahymena* at specific incubation times from shaken and unshaken cultures. Table 1 was obtained from individuals results. Table 2 was obtained from three groups (group Ⅰ, Ⅱ and Ⅲ) which consists of 50 persons.

Table 1 and 2 showed that the population numbers in shaken culture increased more than those in unshaken cul-

Table 1. The average population numbers of *Tetrahymena* cells in the shaken and unshaken cultures (resulted from three individuals A, B, & C)

	Average number of *Tetrahymena* cells per field, corrected for dilution												
	Shaken samples									Unshaken samples			
Hrs \ Count	0	3	6	9	12	15	24	36	48	0	9	15	36
A	3.5	4.0	6.0	11.0	36.0	56.0	76.0	106.0	278.0	1.8	15.1	21.2	34.5
B	4.6	3.1	6.0	65.0	173.0	252.0	114.0	200.0	442.0	18.0	60.0	111.0	185.1
C	2.0	2.4	3.55	33.0	18.0	40.0	556.0	640.0	339.0	2.1	3.9	13.6	33.4
Average	3.4	3.2	5.2	35.3	75.7	116.0	248.7	315.3	353.0	7.3	26.3	48.6	84.3

Table 2. The average population numbers of *Tetrahymena* cells in the shaken and unshaken cultures (from three groups which consist of 50 persons)

	Average number of *Tetrahymena* cells per field, corrected for dilution												
	Shaken samples									Unshaken samples			
Hrs \ Group	0	3	6	9	12	15	24	36	48	0	9	15	36
Group Ⅰ	0.9	2.0	5.2	19.3	29.7	46.7	107.3	200.0	282.0	1.5	4.1	10.3	29.3
Group Ⅱ	3.4	3.2	5.2	35.3	75.7	116.0	248.7	315.3	353.0	7.3	26.3	48.6	84.3
Group Ⅲ	0.1	1.5	6.6	12.5	32.3	53.5	138.3	272.6	379.3	0.1	5.1	9.9	58.3
Average	1.5	2.2	5.7	22.4	45.9	72.1	164.8	262.6	338.1	3.0	11.8	22.9	57.3

ture. After 36 hours incubation, 262.6 cells (group data) and 57.3 cells (individuals data) were obtained from the shaken and the unshaken cultures, respectively. On the basis of data in Table 1 and 2, two kinds of graphs were drawn using arithmetic paper (Fig. 1) and semi-logarithmic graph paper (Fig. 2). In Figure 1, on the ordinary graph paper, average cell numbers (on y-axis) of *Tetrahymena* were plotted against incubation time (on x-axis).
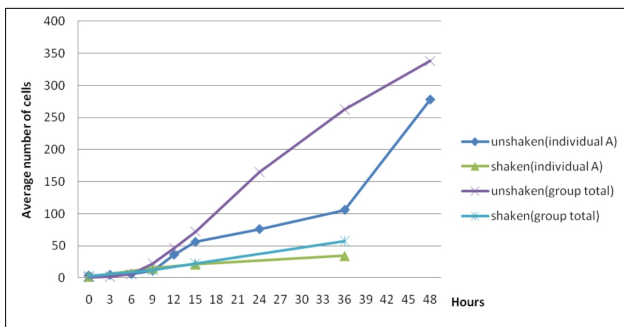
The shaken samples on the arithmetic graph showed

Fig. 1. Population number (N) of *Tetrahymena* vs. incubation time (t) plotted on arithmetic paper.
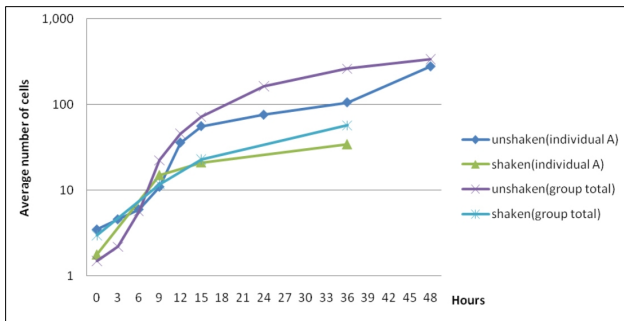

Fig. 2. Population number (N) of *Tetrahymena* vs. incubation time (t) plotted on semi-log paper.

J-shaped curve as a typical exponential growth curve (Fig. 1). The unshaken samples, however, showed a slow increase in population size (N). Using semi-log graph, population growth rates were estimated as the slope of the line according to the different incubation intervals (Fig. 2).

For the comparison of population growth rates (r) between shaken and unshaken samples during different incubation times, r values from both samples were calculated using following equation.

$$r_n = \frac{\ln N_{n+1} - \ln N_n}{t_{n+1} - t_n}$$

In order to calculate the r value (r_1) of the shaken sample (group data) for 0-3 hours incubation time,

$$r_1 = \frac{\ln N_2 - \ln N_1}{t_2 - t_1} = \frac{\ln N_{3\,hr} - \ln N_{0\,hr}}{3-0} = \frac{\ln 2.2 - \ln 1.5}{3}$$
$$= \frac{0.79 - 0.41}{3} = 0.13$$

The r value ($r_1°$) of the unshaken sample (group data) for 0-9 hours incubation time could be calculated as follows.

$$r_1° = \frac{\ln N_2 - \ln N_1}{t_2 - t_1} = \frac{\ln N_{9\,hr} - \ln N_{0\,hr}}{9-0} = \frac{\ln 11.8 - \ln 3.0}{9}.$$
$$= \frac{2.47 - 1.10}{9} = 0.15$$

Table 3 showed that the r values of shaken and unshaken samples of *Tetrahymena* during different incubation periods.

Table 3. The r values of shaken and unshaken samples of *Tetrahymena* during different incubation time intervals

	Shaken								Unshaken		
	r_1	r_2	r_3	r_4	r_5	r_6	r_7	r_8	$r_1°$	$r_2°$	$r_3°$
Time interval (hrs)	0-3	3-6	6-9	9-12	12-15	15-24	24-36	36-48	0-9	9-15	15-36
A	0.05	1.33	0.20	0.39	0.15	0.03	0.03	0.08	0.24	0.06	0.02
Average of group data	0.13	0.32	0.46	0.24	0.15	0.09	0.04	0.02	0.15	0.11	0.04

The r_1 and $r_1°$ represented r values of shaken and un-shaken samples of *Tetrahymena* incubated from initial to 3 hours. Graph 3 and 4 were prepared on the basis of data from Table 3 to study the changing patterns of r values of shaken and unshaken samples according to incubation time.

In shaken samples (Fig. 3), the maximum r value appeared at 6-9 hours incubation time.

As shown in Fig. 4, the r values of unshaken samples decreased continuously along the incubation time. In both samples, r values decreased as the incubation time passed. The r values for the shaken samples were larger than those for the unshaken samples during the majority of the incubation time.

The results of this experiment supported the hypothesis that population growth was faster in shaken samples than unshaken samples. Initially, the growth rate (r) of the shaken samples was increased more rapidly than the un-shaken ones due to aerobic condition. It showed J-shaped curve, as a characteristic of exponential growth, on arithmetic graph paper. The r values of shaken samples at 0-9 hours incubation was increased up to 0.46 but r values of unshaken samples was 0.15. As the incubation time passed (15-36 hours incubation), the growth rate (r) of shaken samples became increased slower and slower due to limiting factors such as density. It seemed to turn out to be a logistic growth which included the carrying capacity (k) of the population growth due to the limiting factors. However, growth rate (r) of the unshaken samples was decreased less than shaken samples due to relatively low density until 15-36 hours incubation time. After 24 hours incubation, growth rates (r) of both samples became almost zero which meant that they approached the plateau as equilibrium state.

DISCUSSION

Tetrahymena pyriformis is used as one of commonly used protozoans in this research of population growth due to its fast growth rate as well as following characteristics. *Tetrahymena* is an oval-shaped and easily observable under the compound microscope with 10× objective lens. It is also reported to be given rapid responses to various experimental conditions, such as oxygen availability, pH, temperature, nutrients, and toxic chemicals (Ward & Codd, 1999; Collins, K, Gorovsky, 2005). This study shows that population numbers (N) and growth rate (r) were larger and faster in the shaken samples (aerobic condition) than in the unshaken samples (anaerobic condition) of *Tetrahymena*. According to this experiment results, an oxygen abundant culture is better condition to growth and reproduction of *Tetrahymena* than an oxygen deficient culture.

The deficiency of oxygen and agitation prevented mating of *Tetrahymena pyriformis*. With oxygen and mixing, 80% of the *Tetrahymena* cells were mated in the laboratory condition (Janetopoulos et al., 1999; Kiersnowska et al., 2000). Janetopoulos et al (1999) reported that *Tetrahymena*
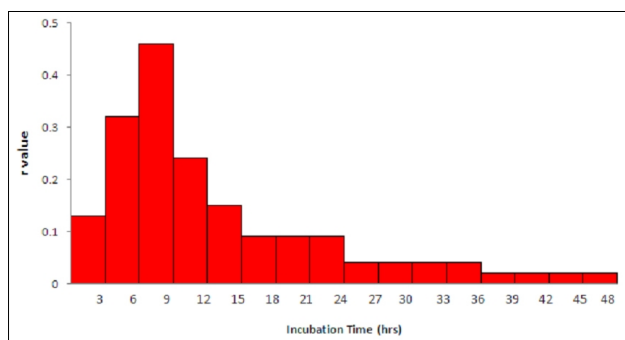

Fig. 3. The r values of shaken samples (individual data) according to the incubation time.
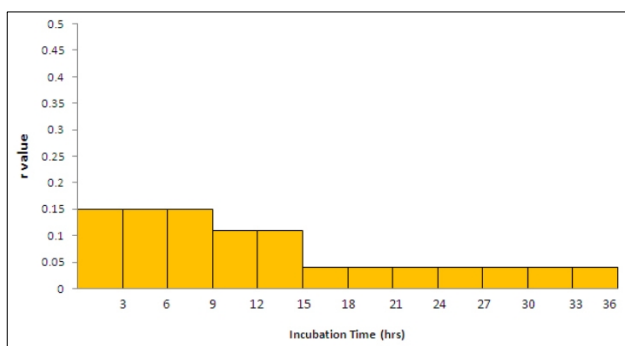

Fig. 4. The r values of unshaken samples (group data) according to the incubation time.

geleii did not grow in size, but did still divide in oxygen concentrations as low as 0.5 mm, whereas the population grew very well at 10 mm oxygen pressure (Collins & Gorovsky, 2005). Therefore, *Tetrahymena* was known to be able to grow under both aerobic and anaerobic conditions.

The population growths of *Tetrahymena* in shaken and unshaken cultures showed both logistic growth models. As shown in Figure 1, during the initial incubation period (0-9 hours incubation), the slope (r) values of shaken samples are steeper than those of unshaken samples. The shaken samples grew more rapidly than the unshaken samples due to abundant resources including oxygen supplement.

In the later incubation period (15-36 hours incubation), unshaken samples grew faster than their initial period because they have relatively abundant resources except oxygen due to slow growth rate of initial phase. However, the shaken samples grew slower than before by depletion of resources due to the increased density. In crowded populations, same as populations of shaken samples in later phase, increasing population density fortifies intraspecific competition for depleting nutrients and other sources resulting in a lower birth and/or growth rates. As population density increases, limiting factors, such as competition, mortality due to starvation, decreased reproduction, and other harmful effects increase. Therefore, the population growth slows down until it stops. If this experiment was extended beyond 48 hours, it is hypothesized that the growth rate (r) of both shaken and unshaken samples would approach to zero (r=0).

In the future, it will be interesting to study effects of oxygen, carbon dioxide concentration, and pH levels on the population size (N) and growth rate (r) of *Tetrahymena*. Pace, D. M. et al reported that the relationship between carbon dioxide as well as oxygen concentration and population numbers of *Tetrahymena geleii* (Collins & Gorovsky, 2005). As CO_2 pressure increased from 0.22 mm CO_2 to 400 mm CO_2 condition, the population density of *T. geleii* was maximal at 0.22 mm CO_2 level(Collins & Gorovsky, 2005). *T. geleii* did not live at high CO_2 concentration (above 122 mm CO_2). According to their results, *T. geleii* grew the best at low CO_2 tension. The future work will be suggested to study the effects of carbon dioxide concentration and pH variation to the population growth rate of *Tetrahymena pyriformis* as following concepts. The varying pH levels will be used by controlling of CO_2 concentration, which is a decreasing factor of pH level of *Tetrahymena pyriformis* culture.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons 2H^+ + CO_3^{2-}$$

The CO_2 will be supplied artificially and also come from respiration of *T. pyriformis*. Then CO_2 reacts with H_2O in culture solution to produce carbonic acid (H_2CO_3). This acid can be ionized to produce protons (H^+), which are the factors of lowering pH level. The pH value can be controlled by pH 0.05 intervals from pH 5 to 9, which include the known optimum pH range (pH 7.25-7.30) (Collins & Gorovsky, 2005). The results will be expected to be two possible cases as shown in Figure 5.

As CO_2 concentration increases, that is, pH level decreases, the growth rate of *T. pyriformis* will increase up to the maximum number, and then decrease dramatically or gradually. In the case of a gradual decrease, it would indicate that the growth of *T. pyriformis* is less sensitive to pH changes (to acidic level). If the growth rate of *T. pyriformis* decreases dramatically, its growth is very sensitive to acidic pH level when it grows and/or reproduces.

This future work can be also suggested that how CO_2 concentration and pH levels as mimic of global warming
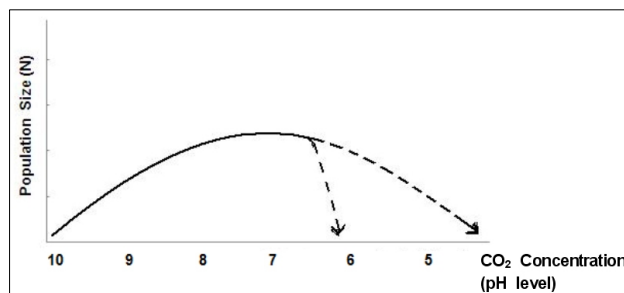

Fig. 5. The expected growth rate profiles of *Tetrahymena pyriformis* by varying CO_2 concentration and pH level.

conditions affect the growth as well as morphology of *Tetrahymena pyriformis*.

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