ZOOLOGICAL SCIENCE 1: 833–836 (1984)

[COMMUNICATION]

Saturation Density of *Tetrahymena* with or without Agitation at Various Surface-to-Volume Ratios of Culture Test Tubes

TADAO SAITOH and HIROSHI ASAI

Department of Physics, School of Science and Engineering, Waseda University, 3–4–1 Okubo, Shinjuku-ku, Tokyo 160, Japan

ABSTRACT — Saturation density of *Tetrahymena pyriformis* in a test tube without shaking can be greatly enhanced, if a small amount of culture medium is layered on top of solid agar containing the same culture medium in the test tube so that the ratio of area of air-medium interface to volume is a large one. Growth of *Tetrahymena pyriformis* in such cultivating tubes with and without shaking was investigated under various conditions of the surface-to-volume ratio. The saturation density of the cells reached 1×10^8 cells/ml in the tube with the largest surface-to-volume ratio ($39\ cm^{-1}$) without shaking. When such a tube was shaken during cultivation, the saturation density of the cells was significantly lower than that without shaking. *T. pyriformis* was also cultivated in a test tube with a small surface-to-volume ratio such that the oxygen supply was insufficient. The saturation density of the cells in such a tube of small surface-to-volume ratio with shaking was slightly higher than that without shaking. These results suggest that the saturation density of the cells in a tube of large surface-to-volume ratio is markedly restricted by slight mechanical agitation (shaking).

We found previously that the lag time of growth of *Tetrahymena pyriformis* was changed by end products from the cells [1]. On the other hand, the end products had no effect on the saturation density of the cells. Namely, the saturation density obtained by cell growth after inoculation in a conditioned medium already containing the end products was almost the same as that obtained by cell growth after inoculation in a fresh medium [1]. Further, in order to elucidate factor(s) restricting the saturation density in a conditioned medium or in a fresh medium, a "crowding" effect involved in the population growth of *T. pyriformis* was investigated by the use of hollow spherical glass beads with a size and weight similar to those of the cells [2]. It was suggested that growth inhibition of *Tetrahymena* under such conditions may be a result of cell-to-cell and cell-to-bead collision. Formerly, it was suggested by some investigators [3–5] that saturation density was dependent upon the amount of oxygen supplied to the culture medium. We had tentatively concluded that the supply of oxygen to the culture medium in culture tubes used in our earlier studies [1, 2] was ample, because the culture tubes were shaken during the culture period in an incubator. However, Dobra *et al.* [6] reported recently that growth of *Tetrahymena* on an agar surface was facilitated by an optimal surface-to-volume ratio, yielding a high density of cells. Furthermore, they mentioned that as cells reached higher densities, their motility decreased. A surface-to-volume ratio of about $1.3\ cm^{-1}$ in the culture tubes was used in our previous studies. Thus, their results could be interpreted as indicating that the culture medium in our culture tubes might not have obtained enough oxygen from air. On the other hand, it could be considered that the reason why the saturation density of *Tetrahymena* cells was very high in their work might have been that the motility of *Tetrahymena* was restricted by the presence of agar. Accordingly, their results could be compatible with our previous proposal with regard to "cell-to-cell collision" as described above. However, it is certain that oxygen supply to the culture medium

Accepted May 2, 1984
Received November 18, 1983

is one of the factors which can restrict the saturation density of growth of *Tetrahymena*.

Thus, in this experiment, the test tubes of various surface-to-volume ratios were used for cultivating *Tetrahymena* cells and measuring their saturation densities at various degree of oxygen supply. We confirmed that the saturation density of *Tetrahymena* cells in a culture tube with a large surface-to-volume ratio is decreased by mechanical agitation even though the oxygen supply is more efficient than it is without mechanical agitation.

MATERIALS AND METHODS

Tetrahymena pyriformis strain W [7] was used in this investigation. The culture medium was composed of 2% proteose peptone (Difco), 0.7% dextrose, 0.5% yeast extract (Wako), 0.5 mM Mg^{2+}, 0.05 mM Ca^{2+}, 25 μg/ml penicillin and 40 μg/ml streptomycin (Kaken) and was adjusted to pH 6.8 with Tris-maleate buffer. The presence of 0.5 mM Mg^{2+} and 0.05 mM Ca^{2+} was required for effective growth, as described in our previous paper [8].

Solution containing the culture medium with 1.5% agar (Shuzui) was boiled and delivered in 5 ml aliquots to 40 culture tubes (3×9 cm, 6.16 cm^2 section area). All the culture tubes containing the solution were stoppered with cotton and autoclaved for 15 min at 120°C and 2 atm. The culture medium in a cotton-stoppered 250 ml Erlenmeyer flask and some pipettes were also autoclaved under the same conditions. For inoculation, 1 ml of stock culture of *Tetrahymena* was transferred to 100 ml of culture medium in the 250 ml Erlenmeyer flask. Aliquots of 0.2 to 10.0 ml of culture medium containing stock cells in the 250 ml Erlenmeyer flask were pipetted aseptically into culture tubes containing solid agar which had previously been formed at room temperature (Fig. 1). This procedure resulted in an initial inoculum of about 10 cells/ml.

Tetrahymena cells in 0.2–10.0 ml of medium were grown in cotton-stoppered upright culture tubes. The solid agar was helpful in preventing the culture medium from evaporating. Twenty of the culture tubes, in which the surface-to-volume ratios were variable, were agitated through an

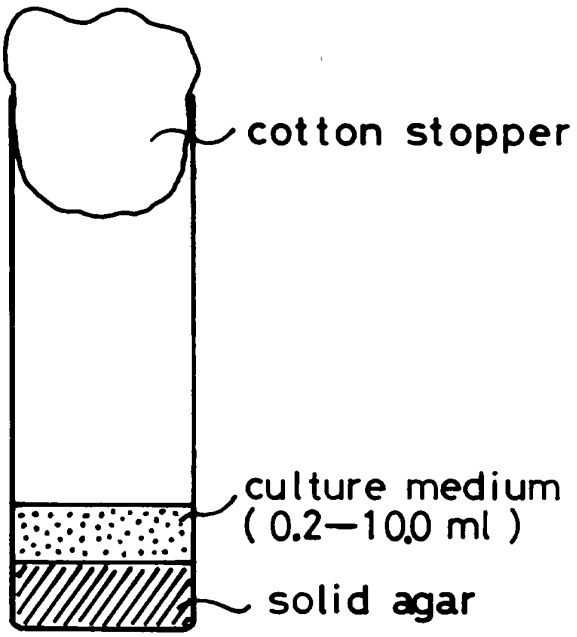

FIG. 1. A test tube (30×90 mm) containing solid agar and culture medium with cotton-stopper for cultivating *Tetrahymena* cells.

excursion of 4 cm at a rate of 40 oscillations/min in an incubator (Taiyo M–100) at 27°C. The other 20 tubes, in which the surface-to-volume ratios were also variable, were not agitated, but were kept in an incubator (Taiyo Mini-80) at 27°C. Each of the incubators was covered with a vinyl bag to prevent the culture medium in the culture tubes from evaporating.

When *Tetrahymena* cells were grown in the culture medium, the generation time at the logarithmic growth phase was about 3.5 hr. The stationary growth phase of the culture having the largest (39 cm^{-1}) surface-to-volume ratio was reached about 6 days after inoculation. The stationary growth phase was maintained for at least 5 more days. For quantitative measurements of the saturation density, a culture medium at 7 days after inoculation was diluted 200–10,000 times with fresh culture medium and then 5 μl of the diluted solution was placed on a glass slide of hematocyte counting chamber. The number of *Tetrahymena* cells on the glass slide was counted under a dark-field microscope. Such dilution and counting was repeated 4 times for each culture medium. After the measurements of the saturation density, the culture medium was transferred to a test tube as a small beaker and then the pH

and volume were measured. The pH and volume of the medium before and after cultivation were constant within 5%.

RESULTS AND DISCUSSION

Culture medium on solid agar was used to cultivate *Tetrahymena* cells as shown in Figure 1. When the culture medium had a large surface-to-volume ratio, the saturation density of the cells reached 1×10^8 cells/ml without shaking. Dobra *et al.* [6] reported that as cells reach higher densities on solid agar, their motility decreases. We also observed under a microscope that the higher the population density of cells in the culture medium was, the lower was their swimming speed, when the culture medium had a large surface-to-volume ratio.

When the cultivation of *Tetrahymena* cells was done in culture tubes with a surface-to-volume ratio of less than 3 cm^{-1}, the saturation density of the cells in a culture tube which had been shaken for 7 days in the incubator was higher than that in a culture tube which had not been shaken. For example, when the cultivation was done at a surface-to-volume ratio of 1.3 cm^{-1}, the saturation densities of the cells in shaken and unshaken culture tubes were 1.2×10^6 cells/ml and 0.7×10^6 cells/ml, respectively (see the inset in Fig. 2). This result suggests that the amount of oxygen supplied to the culture medium in the culture tube was increased by shaking the culture tube and that growth of *Tetrahymena* was promoted.

When the cultivation of *Tetrahymena* cells was done at a surface-to-volume ratio of more than 3 cm^{-1}, the saturation density of the cells in a culture tube which had been shaken for 7 days in incubator was lower than that of the cells in a culture tube

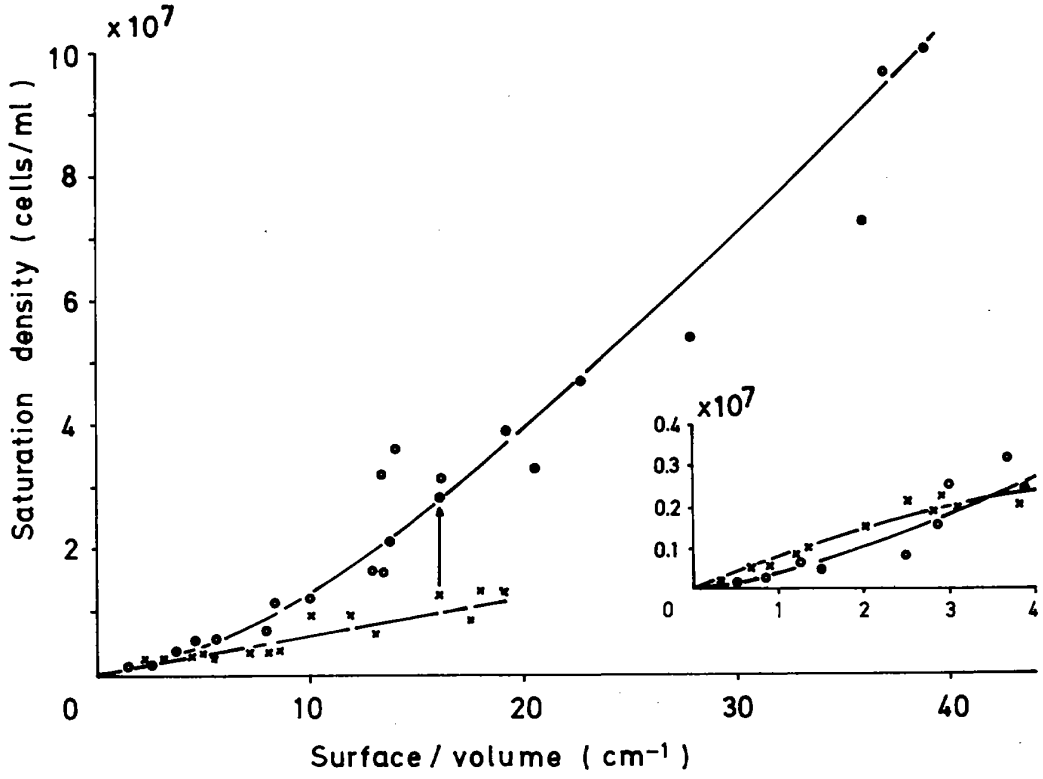

FIG. 2. Saturation densities of *T. pyriformis* grown for 7 days postinoculation in culture tubes containing solid agar, at various surface-to-volume ratios. Saturation densities of *T. pyriformis* in culture tubes with shaking at a rate of 40 oscillations/min (×) and without shaking (○). When *Tetrahymena* cells reached the saturation density under shaking conditions, as shown by an arrow in Fig. 2, the culture tube with a surface-to-volume ratio of 16 cm^{-1} was transferred to an incubator at the same incubating temperature of 27°C for 4 further days without shaking. Its saturation density of *T. pyriformis* reached more than twice that with shaking. Saturation densities of *T. pyriformis* in culture tubes with shaking (×) and without shaking (○) at surface-to-volume ratios of less than 4 cm^{-1} are shown in the inset in enlarged scale.

which had not been shaken. For example, when the cultivation was done at a surface-to-volume ratio of 16 cm^{-1}, the saturation densities of the cells in shaken and unshaken culture tubes were 1.2×10^7 cells/ml and 3.1×10^7 cells/ml, respectively (see Fig. 2). Furthermore, the density of 1.2×10^7 cells/ml in a shaken tube could be increased to 2.8×10^7 cells/ml if the culture tube was further kept without shaking for 4 days in the incubator at 27°C. This suggests that growth of *Tetrahymena* in culture tubes was inhibited by shaking, even though a sufficient amount of oxygen was supplied to the culture medium. The fact that growth of *Tetrahymena* was inhibited by shaking supports the result of our previous investigation [2]. Namely, in our previous paper it was suggested that growth inhibition is a result of cell-to-cell collision, and is not due to the production of waste materials or to exhaustion of nutrients in the medium.

When the cultivation of the cells was done at a surface-to-volume ratio of more than 20 cm^{-1}, the saturation density of the cells reached more than 4×10^7 cells/ml. By slanting, we observed that the culture medium containing the cells had high viscosity.

Hjelm [9] reported that the maximum population density of *Tetrahymena* can reach 1.5×10^6 cells/ml if culture is done in a rotating bottle which provides a large surface-to-volume ratio. This value seems to be at least twice as high as reported for other methods. Hjelm ascribed this to the high oxygen concentration available, though he also mentioned that other factors, such as the accompanying lower CO_2 concentration, may be of importance. Our results indicate that he might have obtained an even higher population density maximum than 2×10^6 cells/ml if the culture bottle had not been rotated, since the oxygen supply should have been ample.

In a quite separate study of *Tetrahymena*, Elliott *et al.* [10] observed that the number and the contents of mitochondria, lysosomes and lipids in *Tetrahymena* were markedly influenced by agitation. Our studies also suggest that physical activity such as cell-to-cell collision has a profound effect on the metabolism of *Tetrahymena* and restricts cell division.

ACKNOWLEDGMENT

We wish to express our thanks to Professor I. Yasumasu of Waseda University for his interest on this work.

REFERENCES

1 Saitoh, T. and Asai, H. (1980) Experientia., **36**: 1375–1376.
2 Saitoh, T. and Asai, H. (1982) Experientia., **38**: 248–249.
3 Pace, D. M. and Ireland, R. L. (1945) J. Gen. Physiol., **28**: 547–557.
4 Levy, M. R. and Scherbaum, O. H. (1965) J. Gen. Microbiol., **38**: 221–230.
5 Malecki, M. T., Licko, V. and Eiler, J. J. (1971) Curr. Mod. Biol., **3**: 291–298.
6 Dobra, K. W., Mcardle, E. W. and Ehret, C. F. (1980) J. Protozool., **27**: 226–230.
7 Nanney, D. L. and McCoy, J. W. (1976) Trans. Am. Microsc. Soc., **95**: 664–682.
8 Saitoh, T. and Asai, H. (1979) J. Protozool., **26**: 286–290.
9 Hjelm, K. K. (1970) Exp. Cell Res., **60**: 191–198.
10 Elliott, A. M., Travis, D. M. and Work, J. A. (1966) J. Exp. Zool., **161**: 177–192.